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Technology in balance with nature

March 28, 2011

Delta Stewardship Council
650 Capitol Mall
Sacramento, CA 95814

Sent via e-mail: deltaplancomment@deltacouncil.ca.gov

Dear Council Members:

Subject: Sacramento Regional County Sanitation District's Comments on the Draft Findings Water Quality, March 9, 2011

The Sacramento Regional County Sanitation District (SRCSD) appreciates the opportunity to provide comments on the attached March 9, 2011 Draft Findings Water Quality (Water Quality Findings). SRCSD is an active participant in finding solutions that will assist the Delta Stewardship Council (Council) in fulfilling its legislative mandates. Our particular area of expertise, and therefore focus, is on water quality. However, there are other issues of relevance and concern to the District as well.

SRCSD believes that the Water Quality Findings will serve as the foundation for the Policies and Recommendations of the Delta Plan, and as such, must clearly document the best scientific understandings of the current state, dynamic properties, and uncertainties of the Delta. As a result, SRCSD believes the Water Quality Findings must be more robust, include complete references and include a much more substantive discussion of the specific information from the references that will be used to scientifically support the Policies and Recommendations of the Delta Plan. For instance, three of the four general findings in this draft Water Quality Findings document do not have references, and are related to drinking water and agricultural beneficial uses. Best available scientific information, its quality, and data gaps should be understood in order to inform the Policies and Recommendations of the second Draft Delta Plan.

SRCSD would like to assist the Council in developing a Delta Plan that is factual, objective, and scientifically based. We are providing the attached specific comments and detailed scientific comments for the Council's consideration in the development of the next draft of the Water Quality Findings Section that will be included in the third Draft Delta Plan. We will be submitting additional comments on the second draft of the Delta Plan in a separate comment letter.

If you have any questions, please contact me at 916-876-6092 or Mitchellt@sacsewer.com.

Sincerely,

Terrie L. Mitchell
Manager, Legislative and Regulatory Affairs

Attachment 1: Draft Findings Water Quality, March 9, 2011
Attachment 2: Specific Comments on Draft Findings Water Quality
Attachment 3: Detailed Scientific Comments Regarding Nutrient Findings

cc: Joe Grindstaff, Executive Officer, Delta Stewardship Council
Dr. Richard Norgaard, Chair, Independent Science Board
Dr. Michael Healey, Vice-Chair, Independent Science Board
Stan R. Dean, District Engineer
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Draft Findings
Water Quality

The State Water Resources Control Board through direct actions and in coordination with the Regional Water Quality Control Boards, known collectively as The Water Boards, regulate point discharges from municipalities, industries, irrigated agricultural lands, and non-point discharges from open lands. The Water Boards issue National Pollutant Discharge Elimination System and Waste Discharge permits for municipalities and industries. These permits are reviewed and renewed periodically. The Water Boards regulate discharges from irrigated agricultural lands under the Irrigated Lands Regulatory Program. The Regional Water Quality Control Boards have issued conditional waivers of waste discharge requirements to growers that have not caused water quality objectives and do not require water quality monitoring.

Water quality in the Delta, especially salinity, is impacted by climatic conditions (freshwater inflows and drought cycles), upstream and in-Delta uses, tidal influences, and in-Delta and export diversions and operations. Water quality is better in the north Delta than in the central and southern Delta because the inflow in the Sacramento River is greater than from the streams that enter the Delta on the east (Cosumnes, Mokelumne, and Calaveras rivers) and in the San Joaquin River and because of agricultural drainage into the San Joaquin River. The State Water Resources Control Board has listed the Delta and San Francisco Bay as impaired under Section 303(d) of the Federal Clean Water Act due to a number of contaminants, including organophosphate and pyrethrin pesticides, elemental mercury, methyl mercury, selenium and unknown toxicity. Other water quality issues within the Delta include salinity, bromide, dissolved organic carbon compounds, nutrients, dissolved oxygen, diazinon, chlorpyrifos, pathogens, polychlorinated biphenyls, turbidity, pharmaceutical residues, and temperature.

In 2010, the State Water Resources Control Board indicated that some of the most serious water quality problems in the Delta watershed and all of California are related to non-point source pollution. Therefore, the Water Boards have prioritized the processes to develop total maximum daily limit criteria on a statewide basis and eliminate the need to develop individual regional criteria. (SWRCB, 2010)

Findings

General

- Future western Delta water quality could reduce beneficial use for drinking water.
- Delta water quality is degraded and could impair beneficial use for drinking water.
- Future western Delta salinity could impair agricultural beneficial use.
- Water quality is degraded and could impair beneficial use for the ecosystem habitat in the future. (CVRWQCB 2007, 2010a, 2010b, 2010d)

Agricultural Discharges

- Pollutants from agricultural discharges have impaired many of California's surface and groundwater resources. (California Water Plan Update 2009, Volume 2, Chapter 17).

Salinity/Salt Management

- Seawater intrusion into the Delta impacts the quality of water exported from the Delta. (California Water Plan Update 2009, Volume 2, Chapter 18)
- California's natural and constructed conveyance systems are not optimized for salt management. (California Water Plan Update 2009, Volume 2, Chapter 18)
- Salt management in California has not kept up with emerging salt problems in many parts of the State. (California Water Plan Update 2009)

Urban Runoff

- Urban runoff presents a threat to both surface and groundwater quality. (California Water Plan Update 2009, Volume 2, Chapter 19)
- Efforts to address urban runoff are most effectively managed at the watershed scale. (California Water Plan Update 2009, Volume 2, Chapter 19)

Nutrients

- Contaminants discharges into the Delta from municipal, industrial, and agricultural sources have affected native species by altering the food webs, reducing food web productivity, and producing toxicity. (Based upon information included in CVRWQCB 2010 Resolution No. R5-2010-0079 and California Review in Fisheries Science 18:211-232, 2010)
- Excessive amounts of ammonium and nitrate, and the ratio of nitrogen to phosphorus, are having a negative effect on the productivity and species composition of phytoplankton in the Delta and stimulate growth of nuisance algae. (Wilkerson et al. 2006, Dugdale et al. 2007, Jassby 2008, and Glibert 2010)

Dissolved Oxygen

- Dissolved oxygen levels drop below water quality objectives at locations within the Delta. (303d list)

Pesticides and Emerging Contaminants

- Most emerging pollutants, such as chemicals found in pharmaceuticals and personal care products, have not been subject to rigorous assessment or regulatory action. (California Water Plan 2009 Update, Volume 2, Chapters 14, 15, and 17)
- New pesticides are approved for use without adequate consideration of potential impacts on aquatic species and ecosystems. (Kuivila and Hladik, 2008, Werner et al, 2008)

Ecosystem Restoration

- Restoring a healthy ecosystem may require developing a more natural salinity regime in parts of the Delta. (Moyle et al. 2010)

Wastewater Infrastructure

- Much of California's wastewater treatment infrastructure has reached or exceeded its useful life expectancy. (California Water Plan Update 2009, Volume 2, Chapter 17).

Climate Change

- Climate change will likely exacerbate existing water quality challenges. (California Water Plan Update 2009, Volume 2, Chapters 14 and 17)

Water Quality Exchanges

- Matching water quality to water use can result in reduced treatment costs and energy consumption. (California Water Plan Update 2009, Volume 2, Chapter 16)

Water Quality Management

- For most water quality contaminants, pollution prevention is more cost-effective than engineered treatment systems. (California Water Plan Update 2009, Volume 2, Chapter, 17)

Attachment Two: Specific Comments on Draft Findings Water Quality

The Sacramento Regional County Sanitation District (SRCSD) is providing the following specific comments by page number and paragraph on the Water Quality Findings. In addition, we have prepared detailed scientific comments on the references used in the nutrient finding and have attached those separately.

Page 1, paragraph 1 – Runoff from irrigated agricultural lands is not a "point discharge" per Clean Water Act (CWA) definition. Point sources include industrial facilities municipal governments and other government facilities (such as military bases). Some agricultural facilities, such as animal feedlots can be defined as point sources, but not irrigated agriculture. "Nonpoint source" pollution is the release of pollutants from everything other than point sources. These include landscape scale sources such as open space and agricultural runoff, and dust and air pollution that find their way into water bodies. Nonpoint source pollution is not typically associated with discrete conveyances. Nonpoint sources are not defined in statute, but are considered everything that is not covered under the point source definition. Use of the point source terminology in the first sentence creates confusion regarding the way such runoff from irrigated agriculture is regulated. The Water Boards issue National Pollutant Discharge Elimination System permits that are to be reviewed and renewed every five years. Waste Discharge Requirements are issued by the Water Boards for public and private facilities, for all types of discharges. The phrase "…have not caused water quality objectives…" in the last sentence of the first paragraph does not make sense. Water quality objectives are not something that is caused. Once established, a regulated discharge either meets the water quality objective or violates it. The conditional waiver of waste discharge requirements under the Water Boards Irrigated Lands Regulatory Program can, and generally does, require water quality monitoring.

Page 1, paragraph 2 – Water quality is better in the North Delta because the Sacramento River dominates this part of the Delta and the quality of the Sacramento River is much better than the San Joaquin for most constituents.

Page 1, paragraph 2 – The 303(d) listings of impaired waters under the Clean Water Act are water body specific. Impairment listings for San Francisco Bay are not appropriately combined with the listings for the Delta. Each water body has its own water quality issues. The water quality issues in San Francisco Bay are in many cases distinct from Delta issues and do not imply impairment in the Delta. The Delta Plan should focus primarily on contaminants of concern in the Delta based on the 303(d) list for the Delta. The Delta Plan should also distinguish between water quality issues in the Stockton Ship Channel as opposed to the remainder of the Delta. In particular, the listings for dissolved oxygen, pathogens and dioxins/furans are specific only to the Ship Channel. When consulting USEPA's 2009 approved 303(d) listing of impaired water bodies for the Delta none are listed as impaired due to nutrients, for any beneficial use.

Delta waters – 303(d) listed parameters [note that not all parameters are listed in all segments of the Delta]

Diazinon
Chlorpyrifos

DDT
Electrical Conductivity (EC)
Mercury
Invasive species
Unknown toxicity
Group A pesticides
PCBs
Chlordane
Dieldrin

Stockton Ship Channel
Above list plus:
Dioxins and Furans
Dissolved oxygen (DO) (TMDL complete and approved)
Pathogens (TMDL complete and approved)

San Francisco Bay – 303(d) listed parameters [note that not all parameters are listed in all segments of the Bay]
Chlordane
DDT
Dieldrin
Dioxins, Furans
Invasive Species
Mercury
PCBs
Selenium
Polycyclic Aromatic Hydrocarbons
Trash

Pyrethroids or pyrethrins are not currently listed in either the Bay or Delta.

Page 1, paragraph 2 – Reference is made to "other water quality issues in the Delta", with a list that includes dissolved organic carbon (DOC), nutrients, pathogens, pharmaceutical residues, DO, turbidity. It is not productive to just list parameters without an explanation of the documented issue. Information is needed to explain the nature of the "issues" and where they exist. Drinking water quality issues are being examined in the Central Valley Drinking Water Policy Work Group (Work Group). Examination of available data at major drinking water intakes has indicated that pathogens are not a current issue; the impact of DOC on water treatment plants is being examined intensively by the Work Group and is not confirmed to be a significant issue; the role of nutrients in taste and odor episodes or nuisance algae blooms has not been clearly established and the significance of the impact to drinking water agencies has not been well quantified. Pharmaceutical "residues" are an identified concern but are not in the category of being an established water quality problem. DO is not an issue in the main channels of the Delta, with the exception of the Stockton Ship Channel, where it is 303(d) listed and a TMDL has been adopted. The issue with turbidity is not clear. Is turbidity an issue because it is decreasing?

Page 1, paragraph 3 –What does the phrase "total maximum daily limit criteria" refer to? Also, the phrase "Eliminate the need to develop individual regional criteria" is used and is referenced to SWRCB (2010). It is unclear what these phrases are intended to mean. What is the title of the SWRCB reference? Clearly there are different regional water quality issues and they should not be addressed the same everywhere in California. What may be a water quality issue in one part of the state is not necessarily an issue in another part of the state. The purpose of having Regional Water Quality Control Boards with individual Basin Plans is to address the regional nature of water quality issues.

Page 1, Findings

First bullet – "Future western Delta water quality could reduce the beneficial use for drinking water" – Is this referring to the possible effects from sea level rise, earthquakes and/or climate change? What is the reference for this statement?

Second bullet - "Delta water quality is degraded and could impair beneficial use for drinking water" What is the basis for this statement? What is the reference for this statement? No 303(d) listings currently exist in the Delta for MUN – Also, the specific nature of impairment is unclear and potentially inaccurate - see comments above regarding DOC, nutrients, pathogens.

Third bullet - "Water quality is degraded and could impair beneficial use for the ecosystem health in the future" (RWQCB refs dated 2007, 2010 a, b and c). Generalized statements such as this are not useful in the management of the Delta. Problem statements need to be specific as to nature, location, etc. to lead to productive solutions. What is this reference?

Page 2, Nutrients

The statement is made that "Contaminants discharges into the Delta from municipal, industrial and agricultural sources have affected native species by altering the food webs, altering food web productivity, and producing toxicity (Resolution R5-2010-0079 and California Review in Fisheries Science, 18:211-232, 2010)" The referenced Water Board resolution generally describes ongoing efforts, such as the development of numeric nutrient criteria for the Delta, and found the cause of the POD investigations in the Delta "inconclusive". This is not a document that presents any evidence of contaminants or nutrients adversely affecting the Delta.

The statement is also made that "Excessive amounts of ammonium and nitrate, and the ratio of nitrogen to phosphorus are having a negative effect on the productivity and species composition of phytoplankton in the Delta, and stimulate growth of nuisance algae" (Wilkerson, 2006, Dugdale 2007, Jassby 2008, and Glibert 2010). These statements are contradicted by statements made by the Central Valley Regional Board in the SRCSD NPDES permit record. What is the specific reference taken from Jassby? The paper by Jassby identifies concerns for ammonium that have been expressed by others, but offers no proof of the adverse effects contained in this statement.

We provide detailed responses to the above statements pertaining to nutrients shown in Attachment 3.

There is confusion between the sub-heading "Nutrients" and the use of "contaminants" in this finding. These terms typically have more specific meanings and it is confusing when they are used interchangeably. "Contaminants" is a generic term indicating a chemical or compound released into the environment that causes an adverse effect. Contaminants may include multiple classes of chemicals. It would be more correct for the heading to be "Contaminants" and nutrients would be one of the classes of contaminants (along with pesticides, CECs, metals, etc.).

Page 2, Dissolved Oxygen

The statement is made that "Dissolved oxygen levels drop below water quality objectives at locations within the Delta" (303(d) list) Dissolved oxygen problems are confined to the Stockton Ship Channel, not to other Delta channels or tributaries to the Delta. This statement should be made specific to the Stockton Ship Channel.

Page 2, Pesticides and Emerging Contaminants

The statement is made that "Most emerging pollutants, such as chemicals found in pharmaceuticals and personal care products, have not been subject to rigorous assessment or regulatory action. (California Water Plan 2009 Update, Volume 2, Chapters 14,15, and 17)" – This finding would greatly benefit from a more substantive discussion of why there has not been a regulatory action taken. The AWWA Research Foundation report on Removal of EDCs and Pharmaceuticals in Drinking and Reuse Treatment Processes (AWWA, 2007) notes that the presence of a compound does not necessarily mean that it is detrimental to the environment. The toxicological significance of trace occurrence of various microconstituents should be determined to establish a scientific basis for establishing sensible monitoring requirements, treatment goals, and regulatory limits.

The risks posed by the presence of contaminants of emerging concern (CECs) to aquatic organisms and to humans are largely unknown, in part because ambient concentrations are difficult to detect and in part due to the lack of demonstrated effects at ambient levels. Science is only in the initial stages of study to gain a full understanding of the health and environmental impacts of CECs.

Of the limited number of studies conducted on the effects of CEC on human health, no studies have effectively linked low concentrations of CECs to adverse health effects in humans. To date, no studies in the U.S have effectively tied changes in the fish populations to wastewater treatment plant effluents. Many data gaps currently exist that researchers are attempting to address including: linking measures of exposure with adverse (and beneficial) effects; linking adverse effects observed in the laboratory with adverse effects in the field; linking adverse effect at the cellular and organ level to adverse effects in the whole organism; linking adverse effects in individual organisms to adverse effect in populations, and evaluating the effect of mixtures of low-concentration microconstituents.

For pesticides, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) provides for federal regulation of pesticide distribution, sale, and use. All pesticides distributed or sold in the United States must be registered (licensed) by EPA. Before EPA may register a pesticide under FIFRA, an applicant must show that using the pesticide according to specifications "will not generally cause unreasonable adverse effects on the environment.'' FIFRA defines the term ''unreasonable adverse effects on the environment'' to mean: '' (1) any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide, or (2) a human dietary risk from residues that result from a use of a pesticide in or on any food inconsistent with the standard under section 408 of the Federal Food, Drug, and Cosmetic Act.''

Additionally, the Endangered Species Act (ESA) of 1973 prohibits any action that can adversely affect an endangered or threatened species or its habitat. In compliance with this law, EPA is required to ensure that use of the pesticides it registers will not harm these species or habitat critical to endangered species survival.

To the extent necessary to prevent unreasonable adverse effects on the environment, the EPA may, by regulation, limit the distribution, sale, or use in any State of any pesticide that is not registered under this Act and that is not the subject of an experimental use permit under section 5 or an emergency exemption under section 18.

USEPA FIFRA regulations are the appropriate manner to regulate pesticides, not through a regulatory body without authority over the registration, sale, use, or distribution of a pesticide. Coordination amongst pesticide regulators and water quality regulators is necessary.

Page 3, Wastewater Infrastructure

The statement is made that "Much of California's wastewater treatment infrastructure has reached or exceeded its useful life expectancy" (California Water Plan Update, Volume 2, Chapter 17) – This statement is inaccurate and misleading.   This statement implies that wastewater treatment facilities are insufficient and in decline. The next sentence from the reference states "Without continued upgrade and replacement, the failure rates of wastewater treatment facilities could increase…" – In fact, starting with the widespread Publically Owned Treatment Works (POTW) construction that occurred in the past 25 to 35 years under Clean Water Grant program, local communities have continued to maintain, upgrade and replace equipment at those facilities.   As a result, most facilities are not at their useful life and are not on the brink of failure due to lack of adequate maintenance or replacement practices.

Page 3, Water Quality Exchanges

The statement is made that "Matching water quality to water use can result in reduced treatment costs and energy consumption" (CWP update, Vol 2, Ch 16) – Is this statement aimed at the encouragement of water recycling?

Page 3, Water Quality Management

The statement is made that "For most WQ contaminants, pollution prevention is more cost-effective than engineered treatment systems" (CWP update, Vol 2, Ch 17) – With regard to wastewater treatment, this statement is misleading, since the ability to meet stringent effluent limits and other permit requirements typically requires treatment. Pollution prevention within a wastewater service area is a typical first step practiced by communities but is rarely the full answer. Does the phrase "engineered treatment systems" pertain specifically to systems to manage municipal, industrial and agricultural sources of contaminants?

Attachment Three: Detailed Scientific Comments Regarding Nutrient Findings

While there is not agreement amongst the scientific community on the degree nutrients and emerging contaminants may be affecting the Delta ecosystem, there is a strong agreement amongst water supply interests that beneficial uses are impaired due to water quality in the Delta. In actuality there has only been a series of hypotheses advanced regarding nutrients potential effects on aquatic life in the delta. Agencies and interested parties have energetically funded research addressing these hypotheses which has been repeatedly evaluated at workshops, by independent panels, and through various State and federal processes that are currently underway.

None of the independent reviews have revealed a consensus that nutrients are a key driver of ecological problems in the Delta, including the pelagic organism decline. Indeed, and despite suggestions by Regional Board staff that there is some type of consensus around effects of ammonia at low concentrations in the Delta, these are only hypotheses. The State Board itself examined the issue just last year, convened an "other stressors" panel in connection with its informational proceeding on Delta flow issues, and concluded only that more study is appropriate.[1]

A finding from an oral presentation (Teh et al. 2009),[2] that ten percent mortality occurred to both *E. affinis* and *P. forbesi* at ambient concentrations present in the river below the SRWTP, is used to suggest that there is a potential for acute ammonia toxicity for Delta copepods. This interpretation is contrary to the Central Valley Regional Board staff interpretations of these same results. In reviewing these test results, Dr. Chris Foe noted that the test pH associated with toxicity in Dr. Teh's experiments (i.e., 7.2) was not representative of ambient pH levels in the Sacramento River (Foe 2009).[3] In his summary, Dr. Foe states that:

> *"Ten percent mortality occurred to both species at ambient ammonia concentrations present in the river below the SRWTP. <u>However, toxicity was only observed at a lower pH (7.2) than commonly occurs in the River (7.4 to 7.8).</u> **Toxicity was not observed when toxicity testing was done at higher pH levels.**"* (Foe 2009, p. 2; emphasis added)

When environmentally representative pH is considered, test results using *E. affinis* and *P. forbesi* do not indicate a potential for acute toxicity in the Sacramento River or the Delta. The LC10s[4] for *E. affinis* and *P. forbesi* at the most environmentally relevant test pH used (pH 7.6) were both about 5 mg N/L total ammonia.[5] This concentration (5 mg N/L) is more than five times higher than the maximum concentrations observed in the Sacramento River during 16 field surveys

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[1] State Water Resources Control Board (2010) Development of Flow Criteria for the Sacramento-San Joaquin Delta Ecosystem. August 3, 2010 (SWRCB 2010); see also District's October 2010 Comments and Evidence Letter, pp. 19-20.

[2] Teh, S., S. Lesmeister, I. Flores, M. Kawaguchi, and C. Teh. 2009. *Acute Toxicity of Ammonia, Copper, and Pesticides to Eurytemora affinis and Pseudodiaptomus forbesi.* Central Valley Regional Water Quality Control Board Ammonia Summit, Sacramento, California, August 18-19, 2009.

[3] Foe, C. 2009. *August 2009 Ammonia Summit Summary*. Technical Memo to Jerry Bruns and Sue McConnell, Central Valley Regional Water Quality Control Board, September 24, 2009.

[4] LC10 is the concentration at which it is estimated there is 10% mortality.

[5] LC10s in Teh et al. (2009) were 5.02 and 5.16 mg N/L total ammonia for *E. affinis* and *P. forbesi*, respectively.

conducted by the Regional Board from 2009-2010 (Foe et al. 2010).[6] Further, the LC10s are higher than the 99.91 percentile of ammonia concentrations occurring in the Sacramento River 350 feet downstream from the SRWTP diffuser.[7] In other words, for all practical purposes, ambient concentrations of total ammonia in the Sacramento River do not exceed the lowest acute thresholds (LC10s) thus far reported for *E. affinis* or *P. forbesi* for representative pH conditions.

With respect to the rest of the Delta, there is also no evidence currently supporting a claim of acute toxicity for adult or juvenile stages of *E. affinis* or *P. forbesi*. None of the ambient total ammonia values measured by the Regional Board at 24 sites throughout the Delta exceeded the environmentally relevant LC10s for the two copepod species (above) during 16 field surveys conducted 2009-2010; most ambient concentrations were more than an order of magnitude lower than the LC10s (Foe et al. 2010)[8]. When expressed as *un-ionized* ammonia, the environmentally relevant LC10s for the two copepod species (0.08 mg N/L un-ionized ammonia for both species at pH 7.6)[9] are well above the 99th percentile (i.e., 0.014 mg N/L un-ionized ammonia) of measured ambient concentrations for the freshwater Delta for 2000-2010 (Figure 1).[10] None of the Regional Board's measurements of total ammonia in the Delta during 2009-2010 (Foe et al. 2010) exceeded the preliminary 96-hour Lowest Observed Effects Concentration (LOEC) for 3-day old nauplii of *P. forbesi* (1.23 mg N/L total ammonia) as reported in a November 10, 2010, letter from Dr. Teh to Dr. Foe,[11] and only one of the ambient un-ionized ammonia measurements in the more extensive dataset illustrated in Figure 1 exceeds the nauplii LOEC when expressed as un-ionized ammonia (0.03 mg N/L un-ionized ammonia at reported test conditions of pH 7.8 and temperature 20°C). Thus, when acute effects thresholds for environmentally representative pH values are compared to ambient ammonia concentrations in the Delta, there is no evidence of acute toxicity to sensitive Delta species.

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[6] Foe, C., A. Ballard, and S. Fong (2010) Nutrient Concentrations and Biological Effects in the Sacramento-San Joaquin Delta. Central Valley Regional Water Quality Control Board, July 2010.

[7] Larry Walker Associates. 2009 Anti-Degradation Analysis for Proposed Discharge Modification to the Sacramento Regional Wastewater Treatment Plant, DRAFT; prepared for Sacramento Regional County Sanitation District, May 2009.

[8] Foe et al. (2010), *supra*

[9] Teh, S., S. Lesmeister, I. Flores, M. Kawaguchi, and C. Teh. 2009. *Acute Toxicity of Ammonia, Copper, and Pesticides to Eurytemora affinis and Pseudodiaptomus forbesi.* Central Valley Regional Water Quality Control Board Ammonia Summit, Sacramento, California, August 18-19, 2009.

[10] Engle, D. (2010) Testimony before State Water Resources Control Board Delta Flow Informational Proceeding. Other Stressors-Water Quality: Ambient Ammonia Concentrations: Direct Toxicity and Indirect Effects on Food Web. Testimony submitted to the State Water Resources Control Board, February 16, 2010.

[11] California Regional Water Quality Control Board, Central Valley Region, Order No. R5-2010-0114 NPDES NO. CA0077682, p. J-3.

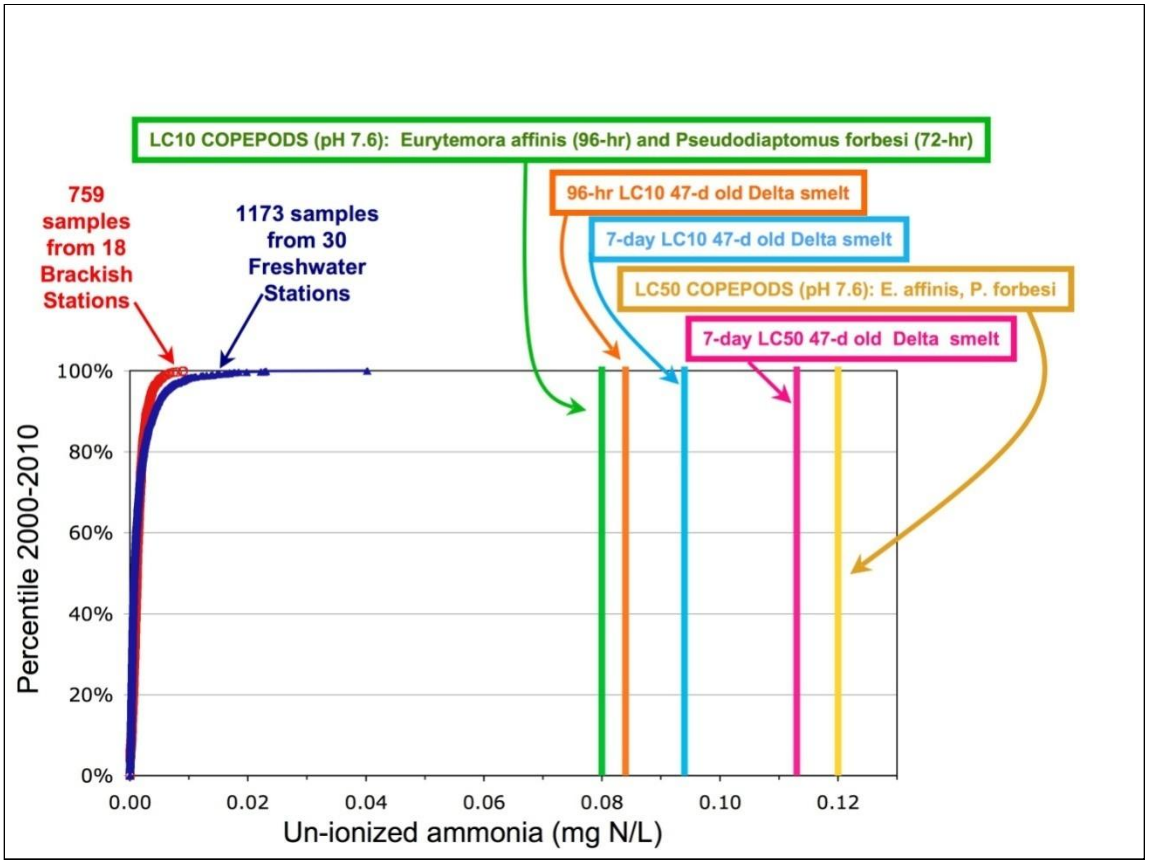

Figure 1. Ranked distribution of ambient concentrations of un-ionized ammonia from estuarine stations (red circles) and freshwater stations (blue triangles) in the upper San Francisco Estuary for 2000-2010. Included are acute effects thresholds for un-ionized ammonia from exposure tests using delta smelt and the adult copepods *Eurytemora affinis and Pseudodiaptomus forbesi*. Preliminary 96-h LC10 for juvenile copepods (3-day-old *P. forbesi* nauplii; 0.030 mg N/L un-ionized ammonia, reported in Nov. 2010. Figure is adapted from Engle (2010).[12]

[12] Engle (2010), *supra, footnote 10*. Figure 3 in Engle (2010) was adapted by adding the LC10 and LC50 for *Pseudodiaptomus forbesi* from Teh, S., S. Lesmeister, I. Flores, M. Kawaguchi, and C. Teh. 2009. *Acute Toxicity of Ammonia, Copper, and Pesticides to Eurytemora affinis and Pseudodiaptomus forbesi.* Central Valley Regional Water Quality Control Board Ammonia Summit, Sacramento, CA, August 18-19, 2009.

The state of knowledge regarding algal preferences for ammonium versus nitrate is incorrectly characterized in multiple venues. One such venue is the USEPA's February 2011 Advanced Notice of Proposed Rulemaking (ANPR). The ANPR cites a paper of Dortch (1990)[13] and a case study from the Baltic Sea (Gry et al. 2001)[14] as support for a hypothesis that flagellates and blue-green algae may out-compete diatoms by preferentially using ammonium, compared to other nitrogen sources. However, information in the well cited, detailed review of Dortch (1990) (summarized below) reveals that generalizations about the nitrogen preferences of phytoplankton taxa are inappropriate.

As explained in Dortch (1990), interactions between the uptake and assimilation of ammonium and nitrate by algae are complex, producing a wide range of outcomes that can be demonstrated in growth experiments, including (a) bona fide preference for ammonium (ammonium uptake is faster than nitrate uptake when each is supplied as the sole N source), (b) bona fide preference for nitrate (nitrate uptake is faster than ammonium uptake when each is supplied as the sole N source), (c) ammonium inhibition of nitrate uptake (nitrate uptake is delayed, or slowed, when both compounds are supplied, compared to nitrate uptake when only nitrate is supplied), and (d) nitrate inhibition of ammonium uptake (ammonium uptake is delayed, or slowed, when both compounds are supplied, compared to ammonium uptake when only ammonium is supplied). All of these types of interactions have been documented in the literature – *and individual taxa can exhibit different types of N-uptake behavior in different environmental conditions*.

Although specific ammonium concentrations are sometimes cited as thresholds for inhibition of nitrate uptake by phytoplankton, little is known about how ammonium/nitrate interactions – and thresholds for interactions – differ among taxonomic classes of phytoplankton. There is a large and sophisticated literature concerning interactions between the uptake and assimilation of nitrate and ammonium by marine and freshwater phytoplankton (Dortch, 1990). The literature reviewed by Dortch indicates that *several factors determine which kinds of nitrogen uptake interactions will be observed for a particular phytoplankton taxon under particular environmental or experimental conditions*. The nitrogen status of algal cells (are they N-limited or N-sufficient?), the N exposure history (*preconditioning*) of algal cells (have they been in a high nitrate, high ammonium, or other type of nitrogen environment?), light levels, and water temperature all influence whether ammonium inhibits nitrate uptake at a given place and time in the lab or in nature (Dortch et al., 1991; Lomas & Glibert, 1999)[15]. Such factors play a role in N uptake kinetics because they affect the mechanisms of transport of compounds across cell membranes, ratios of nitrogen compounds inside cells, and intra-cellular or extra-cellular supplies of enzymes, such as nitrate reductase, urease, and amino acid oxidase. In addition, there is growing evidence that many species of marine and freshwater phytoplankton are also able to utilize amino acids, amides, urea, humic substances, and other dissolved organic nitrogen (DON)

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[13] Dortch, Q. 1990. The interaction between ammonium and nitrate uptake in phytoplankton. Mar. Ecol. Prog. Ser. 61: 183-201.

[14] Gry et al. 2001. Variability in inorganic and organic nitrogen uptake associated with riverine nutrient input in the Gulf of Riga, Baltic Sea. Estuaries 204: 204-14.

[15] Dortch, Q., P. A. Thompson, and P. J. Harrison. 1991. Short-term interaction between nitrate and ammonium uptake in *Thalassiosira pseudonana*: effect of preconditioning nitrogen source and growth rate. Mar. Biol. 110: 183-193.

Lomas, M.W., and P. M. Glibert. 1999. Interactions between NH_4^+ and NO_3^- uptake and assimilation: comparison of diatoms and dinoflagellates at several growth temperatures. Mar. Biol. 133: 541-551.

compounds as sources of nitrogen (Bronk et al., 2007)[16]. DON uptake has been shown to satisfy up to 80% of the total measured N uptake by coastal phytoplankton assemblages.

Enzymatic disruption of nitrate reductase during ammonium assimilation is one of the proposed mechanisms for true "ammonium inhibition". Dortch (1990) explains that, strictly speaking, ammonium inhibition can be demonstrated only when specific uptake rates for nitrate (V_{NO3}) are measured in the presence *and* absence of ammonium, which is not feasible in field experiments or when ambient water containing both forms of DIN is used to measure V_{NO3} or V_{NH4} in the laboratory setting. Many reports of ammonium inhibition in the literature (<u>including Dugdale et al. 2007 and Wilkerson et al. 2006</u>) result from experiments which are not properly designed to distinguish ammonium *preference* from ammonium *inhibition*. Also, inhibition generally varies inversely with the degree of nitrogen deficiency. In other words, phytoplankton that are not N-limited are less likely to exhibit ammonium inhibition of nitrate uptake. This is potentially an important factor influencing ammonium/nitrate interactions in the Delta, which is not considered a nutrient limited environment.

Other environmental factors which control phytoplankton biomass in the Delta greatly constrain the potential effect of ammonium inhibition on overall productivity. Historical data indicates that prior to the arrival of the invasive clam *Corbula amurensis*, June-September were the months of highest mean phytoplankton biomass in Suisun Bay and the western Delta (the confluence zone) (Figure 2). Owing to the overwhelming and well-documented impact of benthic grazing by *Corbula* on phytoplankton biomass during the summer and fall in the brackish Delta (Alpine & Cloern 1992, Jassby et al. 2002, Kimmerer 2005, Thompson 2000)[17], a return of historic summer-fall phytoplankton biomass in the brackish Delta is not expected as long as the estuary remains colonized by *Corbula*—regardless of other physical or chemical changes that may occur in the estuary. Currently, the hypothesized potential for increased diatom biomass in the western Delta related to ammonia reduction is primarily constrained to the April-May window when lower benthic grazing rates (clam grazing), increased water temperature, density stratification, appropriate residence times, and other factors occasionally provide windows for bloom development. However, as Figure 1 illustrates, the presumption that a lowering of ammonium levels to levels observed during the 1970s-1980s would substantially restore annual phytoplankton productivity is flawed. Historically, spring blooms contributed only a small portion of annual phytoplankton biomass. Regardless of future changes in ammonium concentrations, grazing by *Corbula* during summer and fall months would still prevent a recovery of annual algal biomass to levels that occurred historically in Suisun Bay in the 1970s and early 1980s.

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[16] Bronk, D. A., J. H. See, P. Bradley, and L. Killberg. 2007. DON as a source of bioavailable nitrogen for phytoplankton. Biogeosciences 4: 283-296.

[17] Alpine, A.E., and J.E. Cloern (1992) Trophic interactions and direct physical effects control phytoplankton biomass and production in an estuary. Limnol. Oceanogr. 37:946-955.
Jassby, A.D., J.E. Cloern, B.E. Cole (2002) Annual primary production: patterns and mechanisms of change in a nutrientrich tidal estuary. Limnol Oceanogr 47:698-712.
Kimmerer, W.J. (2005) Long-term changes in apparent uptake of silica in the San Francisco estuary. Limnol Oceanogr 50:793-798.
Thompson, J.K. (2000) Two stories of phytoplankton control by bivalves in San Francisco Bay: the importance of spatial and temporal distribution of bivalves. J Shellfish Res 19:612.

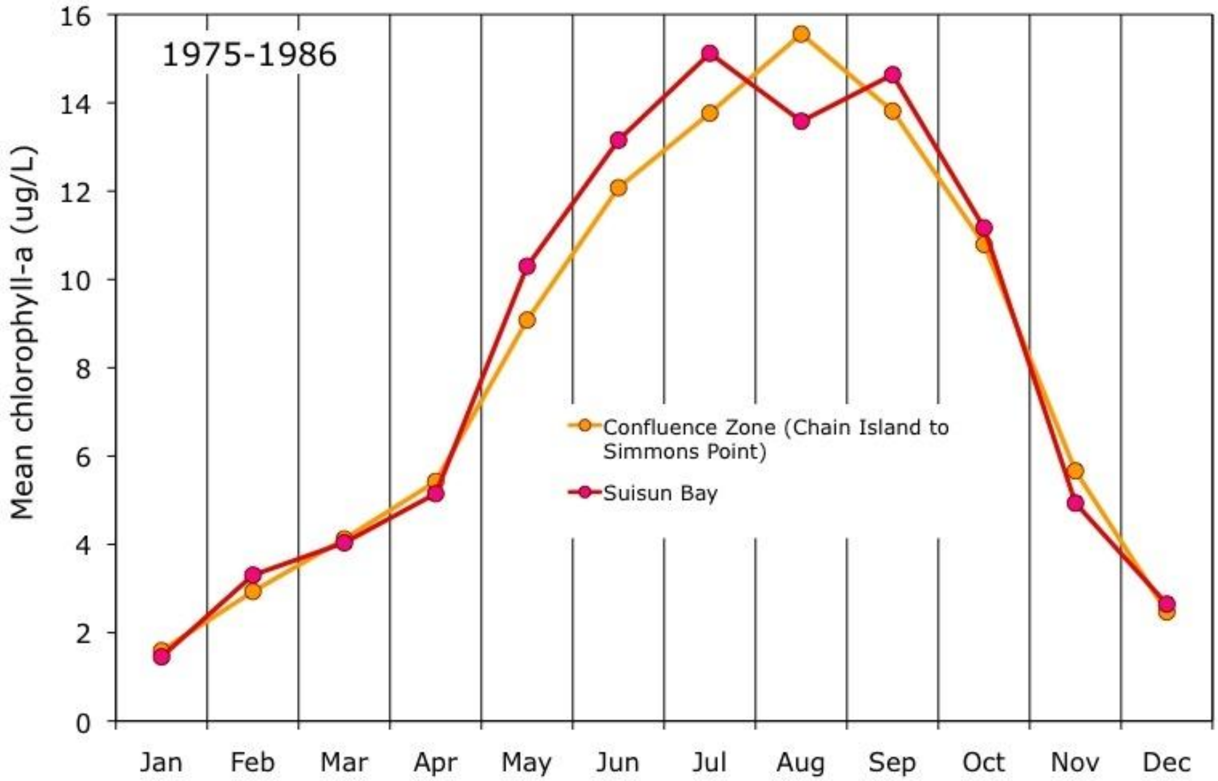

Figure 2. Mean monthly chlorophyll-a concentrations from surface (0.2 m) water samples collected between 1975-1986 at stations used by the IEP, DWR-MWQI, and the USGS. The bulk of annual phytoplankton biomass historically occurred during the same months (June-October) during which *Corbula amurensis* currently controls phytoplankton biomass in the brackish estuary. Figure is from SRCSD (2010).[18]

The water quality findings regarding nutrients cite two publications (Wilkerson et al. 2006[19] and Dugdale et al. 2007)[20] which are commonly cited as evidence that ammonium-induced inhibition of nitrate uptake prevents spring algal blooms from developing in the brackish Delta when conditions are otherwise favorable. However, a critical look at the field data presented in these publications indicates that the ammonium effects observed by these investigators in short, small container experiments do not well predict patterns of phytoplankton biomass in the field. Also, no time series data are presented in either of these publications regarding several environmental parameters (e.g., stratification, benthic grazing rates of clams, herbivorous zooplankton abundance, residence time, Delta outflow) to compare with their records of phytoplankton biomass, although these parameters are critically important to the determination of whether or not conditions are "favorable" for blooms. In the time series data presented in Wilkerson et al. (2006) and Dugdale et al. (2007), algal blooms were evident in Suisun Bay only twice out of

[18] Sacramento Regional County Sanitation District Comments on Draft Nutrient Concentration and Biological Effects in the Sacramento-San Joaquin Delta, Central Valley Regional Water Quality Control Board, May 2010. Letter submitted to Chris Foe, Central Valley Regional Water Quality Control Board, June 14, 2010.

[19] Wilkerson, F.P., R.C. Dugdale, V. Hogue, and A. Marchi. 2006. Phytoplankton blooms and nitrogen productivity in San Francisco Bay. Estuaries and Coasts 29(3):401-416.

[20] Dugdale, R.C., F.P. Wilkerson, V.E. Hogue, and A. Marchi. 2007. The role of ammonium and nitrate in spring bloom development in San Francisco Bay. Est. Coast. Shelf. Sci. 73:17-29.

five periods when ammonium concentrations fell below 4 µM (Figure 3), and one of the blooms (Spring 2003) failed to yield chlorophyll-a levels above 10 µg/L - a level which is frequently (albeit inappropriately, see below) referenced as a threshold for nutritional adequacy for Delta zooplankton (Müller-Solger et al. 2002).[21]

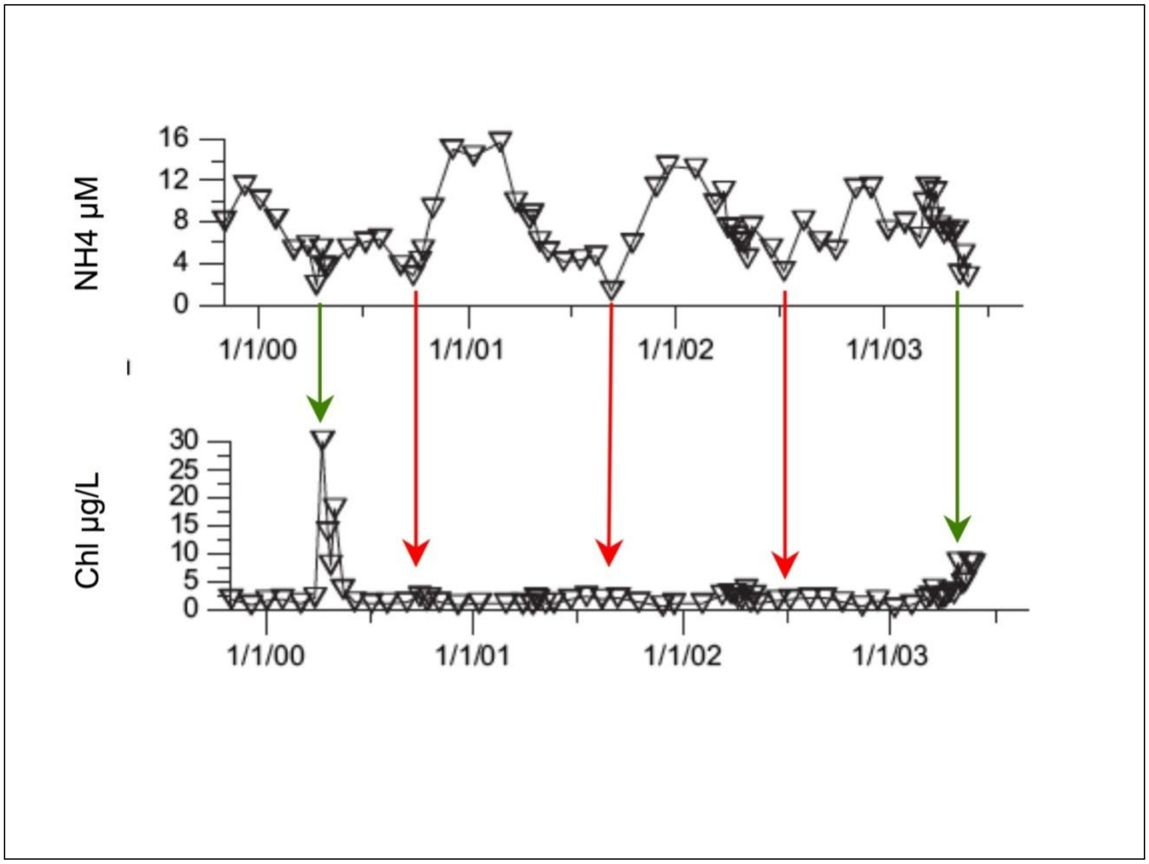

Figure 3. Time series of ammonium and chlorophyll-a from Suisun Bay. Green arrows indicate where ammonium concentrations below a 4 µM threshold were accompanied by increases in chlorophyll-a. Red arrows show periods when similarly low ammonium concentrations were not accompanied by increases in chlorophyll-a. Panels are from Figure 1 in Dugdale et al. (2007); identical time series are presented in Wilkerson et al. (2006).

This lack of consistent correspondence between ammonium concentrations and bloom occurrence amply illustrates that other factors frequently prevent blooms in Suisun Bay, even when ammonium concentrations are below the "Dugdale" threshold of 4 µM. In fact, considering the documented drawdown of ammonium during the onset of blooms by Wilkerson et al. (2006), time series limited to measurements of ammonium and chlorophyll-a cannot rule out the possibility that low ammonium concentrations *in situ* are the *result* of a bloom triggered by non-nutrient factors, rather than the *cause*.
The same methodological shortcomings apply to the recent field work funded by the San

[21] Müller-Solger, A.B., A. D. Jassby, and D. C. Müller-Navarra. 2002. Nutritional quality of food resources for zooplankton (*Daphnia*) in a tidal freshwater system (Sacramento-San Joaquin River Delta). Limnol. Oceanogr. 47: 1468-1476.

Francisco Regional Board, in which ammonia and chlorophyll-a were measured about twice per month during the spring/summer of 2010 - work which has not been made available in a public report, but which was presented at the Bay-Delta Science Conference September 27-29, 2010.[22] The interpretation of field data for ammonia and chlorophyll-a collected on such a coarse time scale – and the absence of accompanying data for other drivers - fails to rule out the possibility that other environmental factors initiate blooms in Suisun Bay, and that low ammonium concentrations are a *result* of the blooms (not a requirement for them).

Ammonia concentrations above the postulated inhibition threshold of 4 µM have been shown to stimulate growth of N-Limited Phytoplankton as they enter the Delta in the Sacramento River. Five-day "grow-out" experiments were conducted by Parker et al. (2010)[23] using Sacramento River water collected above and below the Sacramento Regional Wastewater Treatment Plant (SRWTP) discharge in November 2008, and March and May 2009. The grow-out experiments were intended to control for the effects of light limitation, but by design also eliminated other environmental factors (e.g., gravitational settling and other *in situ* loss factors) that potentially affect riverine phytoplankton biomass in transport through the Delta. During three out of four of the grow-out experiments, phytoplankton grew *better* in water collected at River Mile 44 (below the SRWTP discharge) than they did in Sacramento River water collected above the discharge, even though the ammonium concentrations at River Mile 44 were well above the postulated ammonium inhibition threshold of 4 µM (see Figure 4).[24]

[22] Marchi A., et al. (unpublished data presented at the Bay-Delta Science Conference, Sacramento, CA, September 27-29, 2010).
[23] Parker, A.E., A.M. Marchi, J. Davidson-Drexel, R.C. Dugdale, and F.P. Wilkerson. 2010. Effect of ammonium and wastewater effluent on riverine phytoplankton in the Sacramento River, CA. Final Report. Technical Report for the California State Water Resources Board, May 29, 2010.
[24] Ammonium concentrations in River Mile-44 water used in the grow-out experiments were: July 2008 - 9.06 µM; November 2008 - 71.87 µM; March 2009 - 12.47 µM; May 2009 - 9.54 µM (Table 19-22 in Parker et al. (2010)).

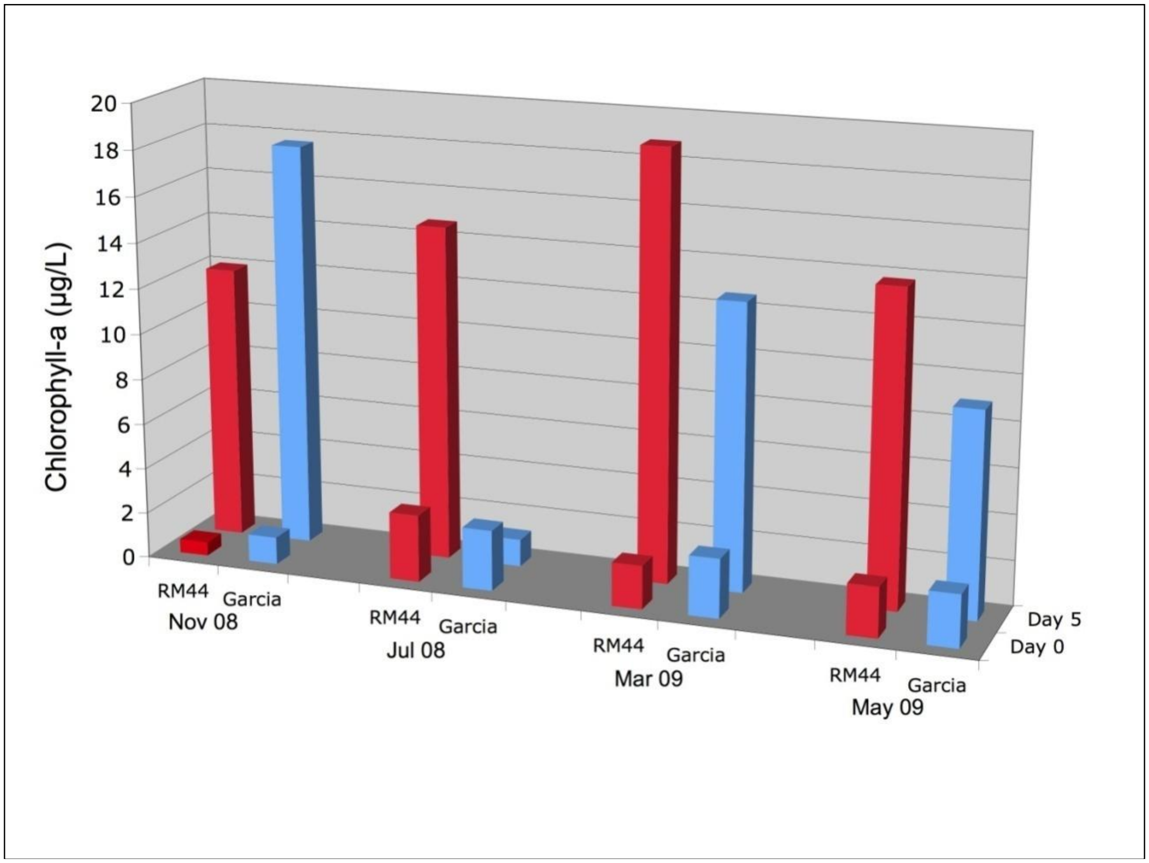

Figure 4. Results of 5-day grow-out experiments using water collected below the SRWTP discharge at River Mile 44 (RM44, red bars) and above the SRWTP discharge (Garcia Bend, blue bars). In three out of four experiments (July 2008, March 2009, May 2009) phytoplankton biomass (chlorophyll-a) was higher after five days in water collected below the SRWTP discharge than in water collected above the discharge. Initial ammonium concentrations in RM-44 water used in the grow-out experiments were: July 2008 - 9.06 µM; November 2008 - 71.87 µM; March 2009 - 12.47 µM; May 2009 - 9.54 µM. Data are from Tables 19-21 in Parker et al. (2010).[25]

These grow-out experiments led Parker et al. to paint a picture of *nitrogen-limited phytoplankton* upstream from the SRWTP, which potentially benefit from the ammonia introduced at the discharge:

> *"Results from experimental grow-outs suggest that after removing light limitation phytoplankton bloom magnitude in the Sacramento River at RM-44 (downstream of SRWTP discharge) and GRC (upstream of SRWTP discharge) is likely determined by dissolved inorganic nitrogen (DIN) availability. Grow-out experiments conducted at RM-44 produced more chlorophyll-a than experimental grow-outs conducted at GRC. Phytoplankton appeared to take advantage of additional DIN, whether supplied as NO3 or NH4 in experiments conducted with water from GRC, or in the form of NH4 supplied in the wastewater effluent (at RM-44) to produce greater biomass*." (Parker et al. 2010, p. 26)

[25] Parker et al. 2010, *supra* note 22

Based on these results, little evidence exists to attribute downstream decreases in chlorophyll-a observed in some field surveys in the Sacramento River to ammonium inhibition, and suggest that it is more appropriate to consider loss factors (e.g., settling) that were nullified by the grow-out tests, but which operate *in situ*.

Longitudinal studies of the Sacramento River contradict claims that ammonium causes a decrease in phytoplankton biomass or primary production rates, or that it changes the cell size or taxonomic composition of phytoplankton in the river. Multiple longitudinal transects measuring nutrients and algal biomass in the Sacramento River from above Sacramento (I-80 bridge) to Suisun Bay were conducted by Regional Board staff (Foe et al. 2010)[26] and Parker et al. (2009, 2010)[27] in 2008-20010. Both studies revealed that although chlorophyll-a often declines in the downstream direction from the I-80 bridge above Sacramento to Rio Vista, no step decline is associated with ammonium inputs related to the Sacramento Regional Wastewater Treatment Plant (SRWTP). For example, in the data shown in Figure 5, more phytoplankton biomass (green line) was lost from river water *above* the SWRTP discharge than below it; and, most of the decline in diatom biomass (blue bars) occurred *upstream* of the SRWTP—a field result which directly contradicts the ammonium-inhibition hypothesis for the Lower Sacramento River portion of the freshwater Delta. Central Valley Regional Board staff have acknowledged that factors unrelated to the SRWTP discharge are needed to explain declines in chlorophyll-a (and other indices of phytoplankton biomass), which were observed between the Yolo/Sacramento County line and the Rio Vista locale during the 2008-2009 field studies:

"The decrease in chlorophyll a appears to commence above the SRWTP. The average annual decline in pigment between Tower Bridge in the City of Sacramento and Isleton is about 60 percent. The cause of the decline is not known, but has been variously attributed to algal settling, toxicity from an unknown chemical in the SRWTP effluent, or from ammonia. The SRWTP discharge cannot be [the] cause of pigment decline upstream of the discharge point, and may not be contributing to the decline downstream of the discharge point." CVRWQCB (2010)[28]

[26] Foe, C., A. Ballard, and S. Fong. 2010. Nutrient concentrations and biological effects in the Sacramento-San Joaquin Delta. Central Valley Regional Water Quality Control Board, Final Report, July 2010.
[27] Parker, A.E., R.C. Dugdale, F.P. Wilkerson, A. Marchi, J. Davidson-Drexel, J. Fuller, and S. Blaser. 2009. *Transport and Fate of Ammonium Supply from a Major Urban Wastewater Treatment Facility in the Sacramento River, CA*. 9th Biennial State of the San Francisco Estuary Conference, Oakland, CA, September 29-October 1, 2009.
Parker et al. 2010, *supra* note 22
[28] California Regional Water Quality Control Board, Central Valley Region, Order No. R5-2010-0114/NPDES NO. CA0077682 page, J-7.

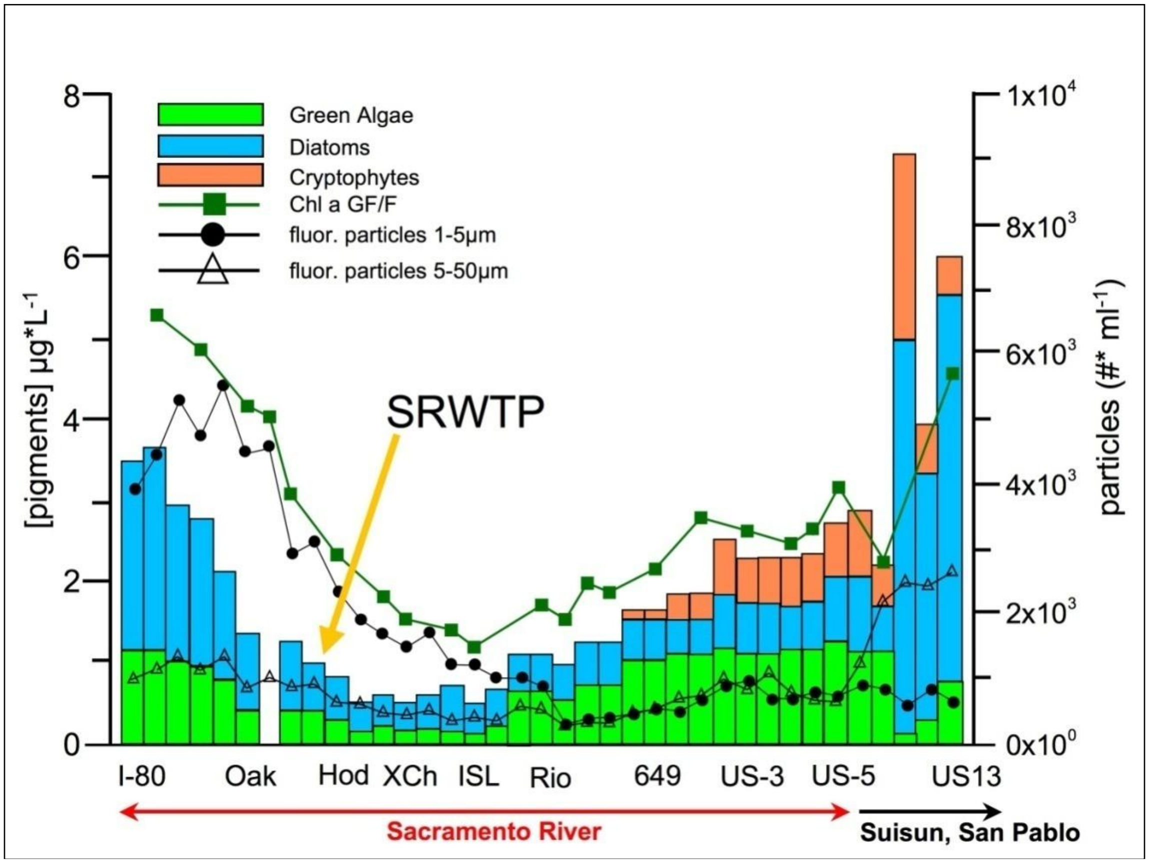

Figure 5. Longitudinal patterns in chlorophyll-a (green squares), biomass of major phytoplankton taxa (colored bars), concentration of small phytoplankton (black circles), and concentration of large phytoplankton (open triangles). Figure is a slight modification from Parker et al. (2009)[29], included in Engle (2010).[30]

Analogous data from Parker et al. (2010)[31] also contradict elements of the ammonium inhibition hypothesis and confirm that the ammonium discharges from the SRWTP cannot explain patterns in phytoplankton biomass, cell size, or taxonomic composition in the Sacramento River. Figure 6 reveals that a downstream decrease in large phytoplankton (assumed by the investigators to be diatoms, shown as light green bars in the figure) is not consistently observed in the river, and when a downstream decrease is observed, it does not begin below the SRWTP discharge. Further, small phytoplankton do not increase in relative abundance below the SRWTP discharge. In fact, the data reveal no consistent longitudinal patterns in the relative abundance of small versus large phytoplankton in the river. In other words, ammonium inputs at the SRWTP discharge do not control the relative abundance of large phytoplankton (presumed to be diatoms) and small phytoplankton. Thus, contrary to the Permit's findings, these field data directly contradict the hypothesis that ammonia will cause small phytoplankton to out-compete large (diatom) phytoplankton.

[29] Parker et al. (2009), *supra*, note 26

[30] Engle, D. (2010) Testimony before State Water Resources Control Board Delta Flow Informational Proceeding. Other Stressors-Water Quality: Ambient Ammonia Concentrations: Direct Toxicity and Indirect Effects on Food Web. Testimony submitted to the State Water Resources Control Board, February 16, 2010.

[31] Parker et al. (2010), *supra* note 22

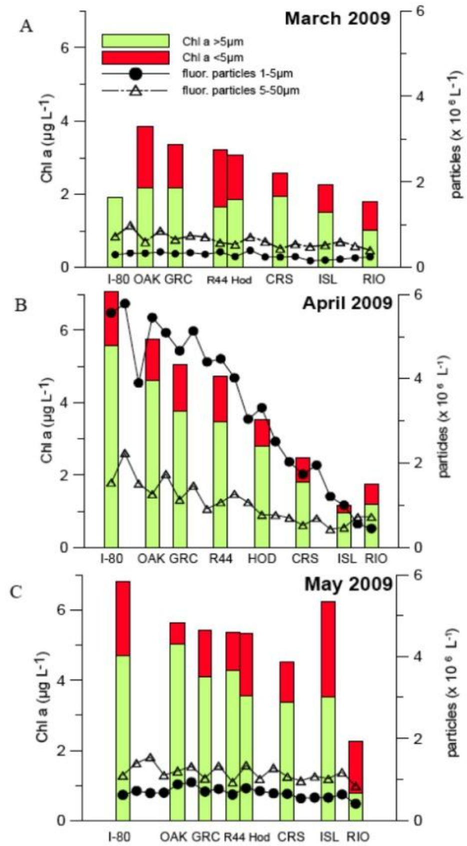

Figure 6. Longitudinal patterns in biomass of large phytoplankton (green bars and open triangles) and small phytoplankton (red bars and closed circles) in the Sacramento River between the I-80 bridge and Rio Vista during Spring 2009; large phytoplankton are presumed by the investigators to include most of the diatoms. Bars indicate biomass as chlorophyll-a; lines indicate cell density measured by fluorescence. Data show that the SRWTP discharge (located between station GRC and R44) does not explain the overall patterns in algal biomass or cell size in the river. Figure is from Parker et al. (2010).[32]

Short-term uptake rate measurements (for carbon, nitrate, and ammonium) made in the same study also contradict elements of the ammonium inhibition hypothesis. Rate measurements in Figure 7 show that primary production rates (black triangles) do not consistently decline in the downstream direction in the Sacramento River, and when they do, the decline is *not* initiated or intensified after water flows past the SRWTP discharge. The field data also clearly show that ammonium uptake rates (see orange symbols in figure) are *not* inversely related to primary production rates (brown triangles in figure) (Parker et al. 2010).[33] Again, these field data directly contradict the hypothesis that ammonium uptake causes a decrease in primary production in the river. These field data clearly demonstrate that predictions about phytoplankton growth

[32] Parker et al. (2010), *supra* note 22
[33] Parker et al. (2010), *supra* note 22

responses and ammonium uptake based on the short-term, small container experiments reported in Wilkerson et al. (2006) and Dugdale et al. (2007) should not be presumed valid outside the laboratory, and cannot be considered evidence of impacts to aquatic life beneficial uses from ammonium in the Delta.

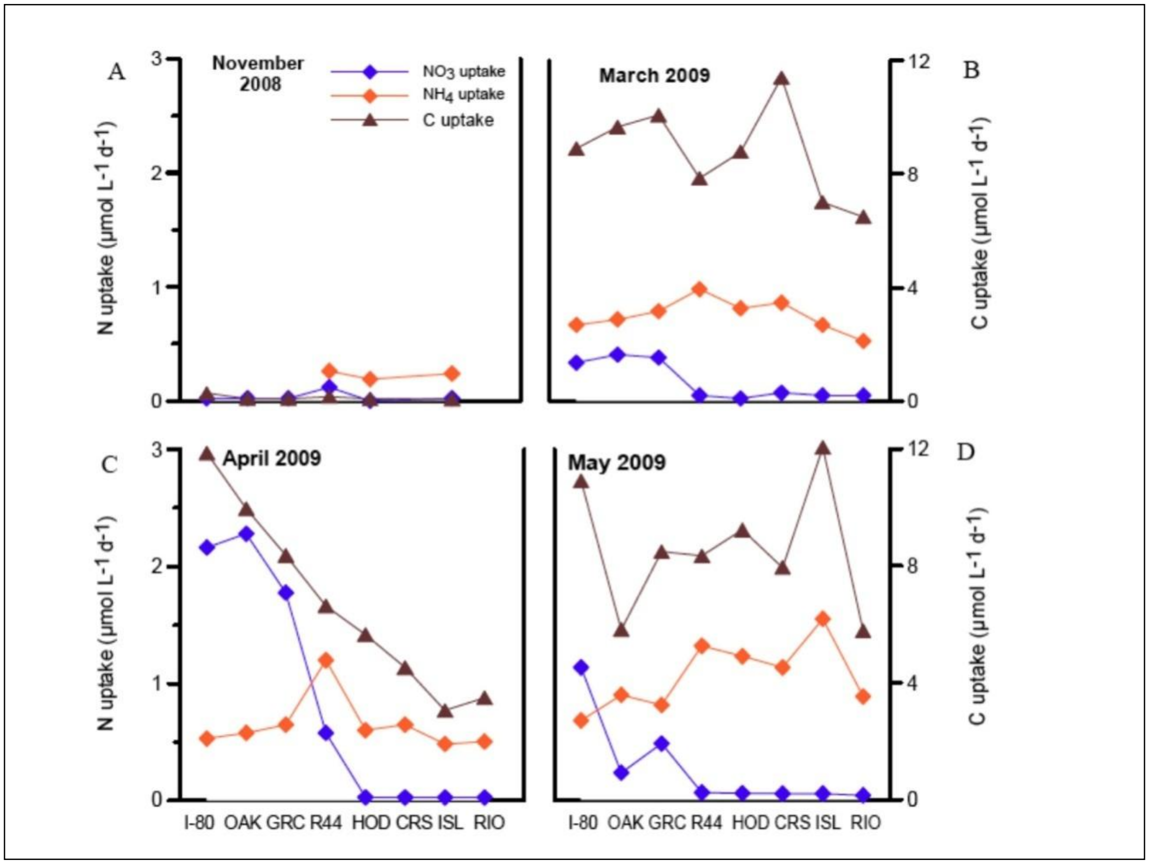

Figure 7. Primary production (C uptake; triangles) and phytoplankton uptake rates of ammonium (orange symbols) and nitrate (blue symbols) made during 24-hr incubations of Sacramento River water collected during four transects between I-80 bridge and Rio Vista. Data do not reveal an inverse relationship between primary production and ammonium uptake. Data further show that longitudinal patterns in primary production are not explained by the SRWTP discharge (located between GRC and R44). Figure is from Parker et al. (2010).[34]

Data from a longer longitudinal transect in the Sacramento River also contradict proposals for an inverse relationship between ammonium uptake and primary production in the Delta. The longitudinal transects by the Parker/Dugdale team during this 2008-2009 Sacramento River project included rate measurements (uptake of carbon, ammonia, and nitrate) at 21 stations starting from I-80 above the city of Sacramento downstream through Suisun Bay and into San Pablo Bay.[35] These rate measurements show a decline in primary production (carbon uptake, indicated by black line in Figure 8) in the upstream reach where nitrate uptake (shown by blue

[34] Parker et al. (2010), *supra* note 22
[35] Parker et al. (2009), *supra*, note 26

bars) exceeded ammonium uptake (shown by red bars). The measurements show that the carbon uptake pattern was independent from the relative contribution of ammonium and nitrate to inorganic nitrogen uptake. Also, in the dataset illustrated in Figure 8, significant *increases* in carbon fixation began in the confluence zone (stations 649 through US3), despite the fact that inorganic nitrogen uptake was dominated by ammonium in that reach. Collectively, these results imply that other factors (probably hydrodynamic factors such as stratification, current speed, residence time) are controlling phytoplankton biomass and primary production in the Sacramento River—not ammonium inhibition.

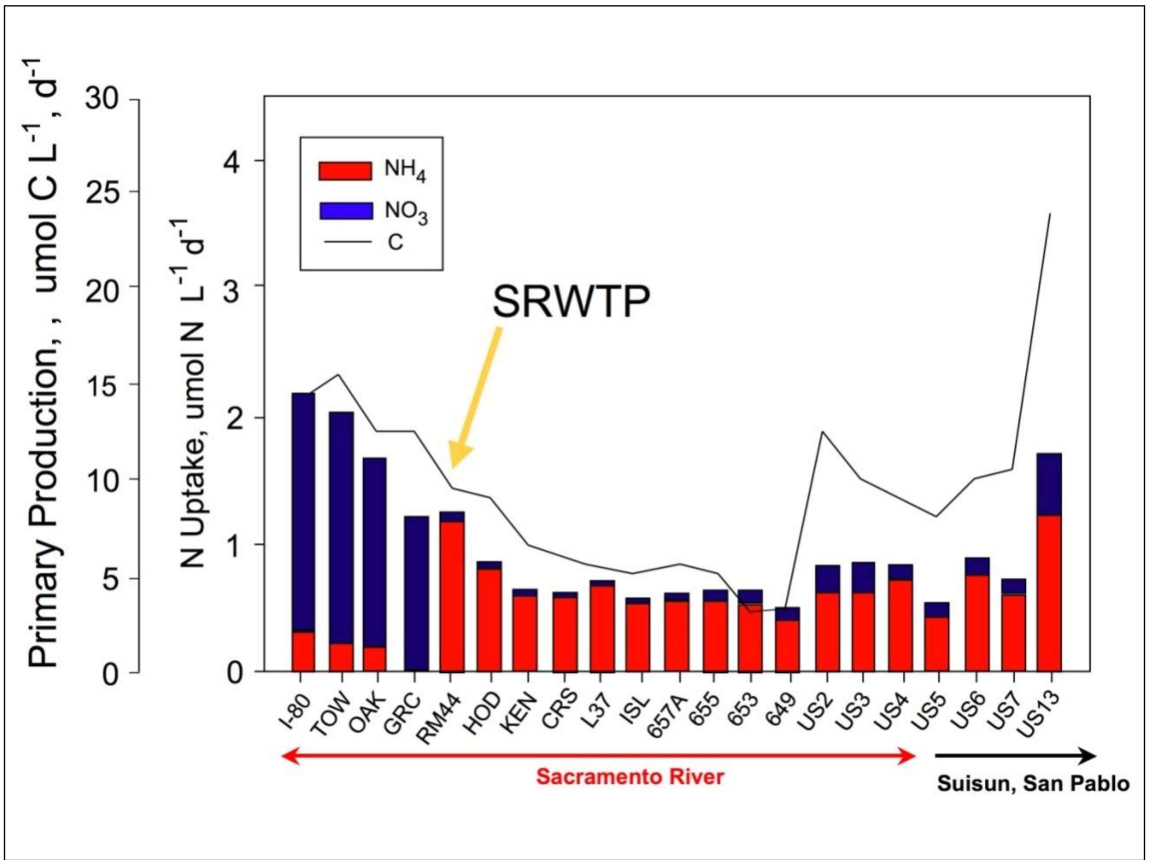

Figure 8. Longitudinal patterns in primary production (black line) and rates of ammonium uptake (red bars) and nitrate uptake (blue bars) in the Sacramento River in March 2009. Data indicate that the location of the SRWTP (and a switch from nitrate to ammonium uptake) does not initiate the decline in primary production in the river, nor does ammonium uptake prevent increases in primary production in the confluence zone (stations 649 through US3).

Evidence from studies conducted in the Delta contradicts the hypothesis that ammonia, or nutrient ratios involving ammonia, promote blooms of microcystis (blue-green algae). Available research from the Delta argues against a simplistic association between *Microcystis* and nutrient form or concentration. Delta studies conducted by Lehman et al. (2008, 2010)[36] and Mioni

[36] Lehman, P.W., G. Boyer, M. Satchwell, and S. Waller. 2008. The influence of environmental conditions on the seasonal variation of *Microcystis* cell density and microcystins concentration in the San Francisco Estuary.

(2010)[37] have found no apparent association between ammonium concentrations or NH_4^+:P ratios and either *Microcystis* abundance or toxicity. Instead, it appears from these studies that water temperature is strongly positively correlated with *Microcystis* abundance and toxicity; and, that water transparency, flows, and specific conductivity are also potential drivers of *Microcystis* blooms in the Delta. Specifically, an association between water temperature and *Microcystis* blooms in the Delta is supported by the upward trend in spring-summer mean water temperature in the freshwater Delta between 1996-2005 (Jassby 2008)[38] and would be consistent with observations from other estuaries, where increased residence time (e.g., during drought) and warmer temperatures are acknowledged as factors stimulating cyanobacterial (i.e., *Microcystis*) blooms (Pearl et al. 2009; Pearl & Huisman 2008; Fernald et al. 2007).[39] In addition, there is evidence from other estuaries, and from studies conducted in the Delta (summarized below), that resistance to grazing by molluscs and zooplankton can confer a selective advantage to *Microcystis* and may operate to enhance or prolong *Microcystis* blooms. For example, selective grazing by the non-native Delta copepod *P. forbesi* was recently demonstrated as a viable mechanism for promoting *Microcystis* blooms (Ger et al. 2010).[40]

Information from the Delta and other estuaries indicates that non-nutrient factors are credible alternative explanations for the observed shift in phytoplankton species composition in the Delta. Physical factors (such as temperature, current speed, residence time, turbulent mixing, stratification, light penetration) may be strongly affecting competitive outcomes between diatoms and other phytoplankton taxa in the Delta; temporal changes in these physical and hydrodynamic factors may be responsible for observed shifts in phytoplankton species composition in the Delta (e.g., fewer diatoms, more blue-greens and flagellates). The influence of flows and residence time on phytoplankton assemblages in estuaries is well-acknowledged in other regions. For example, hydrologic perturbations, such as droughts, floods, and storm-related deep mixing events, overwhelm nutrient controls on phytoplankton composition in the Chesapeake Bay; diatoms are favored during years of high discharge and short residence time.[41] The role of flow and residence time in regulating estuarine phytoplankton composition was summarized by the expert panel convened by CalFed in March 2009 in their final "*Ammonia Framework"* document:

Hydrobiologia 600:187-204.

[37] Mioni, C.E., and A. Paytan. 2010. *What controls Microcystis bloom & toxicity in the San Francisco Estuary? (Summer/Fall 2008 & 2009)*. Delta Science Program Brownbag Series, Sacramento, CA. May 12, 2010.

[38] Jassby, A. 2008. Phytoplankton in the Upper San Francisco Estuary: recent biomass trends, their causes and their trophic significance. San Francisco Estuary & Watershed Science, Feb. 2008.

[39] Pearl, H.W., K.L. Rossignol, S. Nathan Hall, B.L. Peierls, and M.S. Wetz. 2009. Phytoplankton community indicators of short- and long-term ecological change in the anthropogenically and climatically impacted Neuse River Estuary, North Carolina, USA. Estuaries and Coasts. DOI 10.1007/s12237-009-9137-0.

Pearl, H.W., and J. Huisman. 2008. Blooms like it hot. Science 320:57-58. doi:10.1126/science.1155398.

Fernald, S.H., N.F. Caraco, and J.J. Cole. 2007. Changes in cyanobacterial dominance following the invasion of the zebra mussel *Dreissena polymorpha*: long-term results from the Hudson River Estuary. Estuaries and Coasts 30:163-170.

[40] Ger, K.A., P. Arneson, C.R. Goldman, and S.J.Teh. 2010. Species specific differences in the ingestion of *Microcystis* cells by the calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi*. Short Communication. J. Plankton Research. doi: 10.1093/plankt/fbq071

[41] Pearl, H.W., L.M. Valdes, B.L. Peierls, J.E. Adolf, and L.W. Harding, Jr. 2006. Anthropogenic and climatic influences on the eutrophication of large estuarine ecosystems. Limnol. Oceanogr. 51(1, part 2): 448-462.

> *"Diatoms have fast growth rates and may be particularly good competitors during high flows with concomitant short residence times, when their fast growth rates can offset high flushing rates. In moderate flows, chlorophytes and cryptophytes become more competitive, whereas low flows with concomitant longer residence times allow the slower-growing cyanobacteria, non-nuisance picoplankton, and dinoflagellates to contribute larger percentages of the community biomass. These spatially and temporally-variable patterns of phytoplankton composition are typical of many estuaries [e.g., Chesapeake Bay, Maryland; Neuse-Pamlico Sound, North Carolina; Narragansett Bay, Rhode Island; Delaware Bay, Delaware]". (Meyer et al. 2009)*[42]

The idea that flows influence diatom abundance is not new in the Delta. Lehman (1996, 2000)[43] associated a multi-decadal decrease in the proportional biomass of diatoms in the Delta and Suisun Bay to climatic influences on river flow. The Central Valley Regional Board recently found that current speed in the Sacramento River was related to the difference in phytoplankton biomass between Freeport and Isleton (Foe et al. 2010).[44]

Top-down effects on phytoplankton composition, caused by selective grazing by clams and zooplankton, are likely to influence the species composition of phytoplankton in the Delta, and may contribute to the occurrence of *Microcystis*. Clam grazing selectively removes larger particles from the water column (Werner & Hollibaugh 1993);[45] clams may consume a larger fraction of diatoms than smaller plankton taxa such as flagellates. Kimmerer (2005)[46] attributed a step decrease in annual silica uptake after 1986 to efficient removal of diatoms by *Corbula amurensis* after its introduction in 1986. Grazing by *Corbicula fluminea* can cause shallow habitats in the freshwater Delta to serve as a net sink for phytoplankton (Lopez et al. 2006, Parchaso & Thompson 2008)[47]; it is possible that diatoms are differentially affected by benthic grazing (e.g., compared to motile or buoyant taxa) in both the brackish and freshwater Delta.

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[42] Meyer, J.S., P.J. Mulholland, H.W. Paerl, and A.K. Ward. 2009. A framework for research addressing the role of ammonia/ammonium in the Sacramento-San Joaquin Delta and the San Francisco Bay Estuary Ecosystem. Final report submitted to CalFed Science Program, Sacramento, CA, April 13, 2009.

[43] Lehman, P.W. 1996. Changes in chlorophyll-a concentration and phytoplankton community composition with water-year type in the upper San Francisco Estuary. (pp. 351-374) In Hollibaugh, J.T, (ed.) San Francisco Bay: the ecosystem. San Francisco (California): Pacific Division, American Association for the Advancement of Science.

Lehman, P.W. 2000. The influence of climate on phytoplankton community biomass in San Francisco Bay Estuary. Limnol. Oceanogr. 45: 580-590.

[44] Foe, C., A. Ballard, and S. Fong. 2010. Nutrient concentrations and biological effects in the Sacramento-San Joaquin Delta. Central Valley Regional Water Quality Control Board, Final Report, July 2010.

[45] Werner, I., and J.T. Hollibaugh. 1993. *Potamocorbula amurensis*: Comparison of clearance rates and assimilation efficiencies for phytoplankton and bacterioplankton. Limnol. Oceanogr. 38: 949-964.

[46] Kimmerer, W.J. 2005. Long-term changes in apparent uptake of silica in the San Francisco Estuary. Limnol. Oceanogr. 50: 793-798.

[47] Lopez, C.B., J.E. Cloern, T.S. Shraga, A.J. Little, L.V. Lucas, J.K. Thompson, and J. R. Burau. 2006. Ecological values of shallow-water habitats: implications for the restoration of disturbed ecosystems. Ecosystems 9: 422-440.

Parchaso F., and J. Thompson. 2008. *Corbicula fluminea* distribution and biomass response to hydrology and food: A model for CASCaDE scenarios of change. CALFED Science Conference, Sacramento, CA., October, 2008. Avail at http://cascade.wr.usgs.gov/CALFED2008.shtm.

Significantly, benthic grazing has been implicated as a factor favoring *Microcystis* over other phytoplankton, as explained in the CalFed expert panel's "*Ammonia Framework:"*

> *"However, in places where filter-feeding mussels and clams overlap with habitat suitable for Microcystis (i.e., low salinity), the presence of these invertebrates might enhance bloom formation by selectively rejecting large Microcystis colonies. That grazer selectivity can give Microcystis a grazer-resistant, competitive advantage over other phytoplankton, as Vanderploeg et al. (2001) reported for zebra mussels (Dreissena polymorpha) in the Great Lakes."* (Meyer et al. 2009)[48]

In addition to grazing by mussels and clams, grazing by zooplankton can exert a top-down effect on phytoplankton composition; the literature regarding selective feeding by zooplankton is impractical to review herein. However, in a particularly pertinent example, selective grazing by the Delta copepod *P. forbesi* was recently demonstrated as a viable mechanism for promoting *Microcystis* blooms (Ger et al. 2010).[49]

Experimental data from the Delta contradicts the simplistic assumption that the pelagic food web in the Delta is dependent on diatom biomass. The widespread assumption that a decline in the relative abundance of diatoms and an increase in other taxa including flagellates, green algae, and cyanobacteria represents a significant degradation of food resources for primary consumers in the Delta (estuarine mesozooplankton, and calanoid copepods in particular) has not been critically examined in policy and regulatory arenas, and is flawed.
At least six different lines of evidence challenge the simplistic "diatom → copepod → pelagic fish" paradigm that is used to justify much of the attention regarding ammonia and the SFE food web:

1. Diatoms can be toxic to copepods. A large body of literature indicates direct feeding on diatoms can cause reproductive failure in copepods (Ianora & Miralto 2010, and references therein).[50] This potential harmful effect of diatoms on copepods, first described in the early 1990s, prompted an ongoing re-evaluation of the classic paradigm that "diatoms-beget-copepods-beget-fish" and has been the subject of considerable research and special workshops and symposia. The harmful effect is caused by organic compounds (oxylipins), which are released from diatom cells when they are broken during feeding. These compounds then induce genetic defects in copepod eggs. The genetic defects are manifested by a failure of the eggs to hatch or a failure of hatched offspring to develop normally. <u>These toxic effects of diatoms are unrecognized in lab or</u>

[48] Meyer, J.S., P.J. Mulholland, H.W. Paerl, and A.K. Ward. 2009. A framework for research addressing the role of ammonia/ammonium in the Sacramento-San Joaquin Delta and the San Francisco Bay Estuary Ecosystem. Final report submitted to CalFed Science Program, Sacramento, CA, April 13, 2009.
[49] Ger, K.A., P. Arneson, C.R. Goldman, and S.J.Teh. 2010. Species specific differences in the ingestion of *Microcystis* cells by the calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi*. Short Communication. J. Plankton Research. doi: 10.1093/plankt/fbq071
[50] Ianora, A. and A. Miralto (2010) Toxigenic effects of diatoms on grazers, phytoplankton and other microbes: a review. *Ecotoxicology,* 19, 493-511.

field studies from the Delta that rely on gut contents, clearance rates, or egg counts to determine the nutritional status of copepods, or to infer the nutritional value of suspended matter, because the harmful compounds involved in diatom toxicity do not affect feeding behavior or the numbers of eggs produced, but instead affect the viability of the eggs that are produced after feeding. There are at least twenty-four (24) recently published experiments indicating harmful effects of diatom grazing for copepod species pertinent to the SFE (i.e., SFE species and their co-familials) (Figure 9).

2. Delta copepods prefer non-diatom prey. Published experiments from the Delta show that key Delta copepods (including the ones that delta smelt eat) actually prefer *non*-diatom types of phytoplankton and that much of the time they do not consume phytoplankton at all (preferring instead to consume small heterotrophic organisms in the water column)[51]. These feeding experiments indicate that the principal calanoid copepods in the estuary (*Acartia* spp., *E. affinis*, *P. forbesi*) prefer motile prey over non-motile prey, and prefer heterotrophic prey (e.g., cilliates, heterotrophic dinoflagellates) over phytoplankton (Bollens & Penry 2003, Bouley & Kimmerer 2006, Gifford et al. 2007).[52] Diatoms are not motile, as they lack flagella or other means of locomotion. Thus, Delta copepods do not rely on diatoms as a direct food source, and frequently discriminate against phytoplankton altogether (even during diatom blooms), depending on season and location in the estuary.

3. The reproductive implications of food *choices* are virtually unstudied for the copepods of the San Francisco Estuary. A recent review of almost 400 research articles regarding the feeding ecology of copepod taxa in the families occurring in the Bay-Delta revealed that only three published studies measured egg production or hatching success for a Delta-pertinent copepod species fed mixtures of diatoms and non-diatoms (Engle 2010).[53] In other words, there is essentially no science which addresses whether observed changes in phytoplankton composition in the Bay Delta Estuary could have had population-level consequences for copepods.

4. Many non-diatom classes of phytoplankton are highly nutritious. Non-diatom classes of phytoplankton (including some groups which are now more abundant in the estuary) include species that are considered highly nutritious for zooplankton. Examples include cryptophytes (e.g., *Cryptomonas* and *Rhodomonas* spp.) and many species of green algae (e.g., *Scenedesmus* spp.), which are used as food to rear zooplankton in laboratories.

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[51] Heterotrophic organisms (such as bacteria and many protozoa) obtain energy by consuming pre-existing organic matter, as opposed to synthesizing organic matter through photosynthesis.

[52] Bollens, Gretchn C. Rollwagen, Penry, Deborah L. Feeding dynamics of *Acartia* spp. copepods in a large, temperate estuary (San Francisco Bay, CA).
Bouley, P. and W.J. Kimmerer (2006) Ecology of a highly abundant, introduced cyclopoid copepod in a temperate estuary. *Marine Ecology-Progress Series,* 324, 219-228.
Gifford, S.M., G. Rollwagen-Bollens, and S.M. Bollens. (2007) Mesozooplankton omnivory in the upper San Francisco estuary. *Marine Ecology-Progress Series,* 348, 33-46.

[53] Engle, D. (2010) Slides and Oral Remarks Presented in: *Engle, D. (2010) How well do we understand the feeding ecology of estuarine mesozooplankton? A survey of the direct evidence.* 6th Biennial Bay-Delta Science Conference, Sacramento, CA, September 27-29, 2010, 31 pp.

5. The interpretation of a specific chlorophyll-a level as an indicator of nutritional sufficiency for Delta copepods is unjustified. Chlorophyll-a levels below 10 µg/L are frequently cited in Delta literature as evidence that zooplankton in the Delta are food limited (e.g., see Muller-Solger et al. 2002).[54] However, this threshold is based on a set of laboratory growth experiments conducted with a single cladoceran zooplankton species (*Daphnia magna*) and it is unclear whether this threshold is appropriately applied to any of the copepods in this system, especially given the importance of non-phytoplankton particles in the diet of Delta copepods. The heavy reliance of SFE copepods on non-algal foods indicates that detritus-based pathways for energy transfer may contribute more to the pelagic food web in the Delta than has been acknowledged. Such information led the IEP to make the following acknowledgement in its 2007 Synthesis of Results:

   "*. . . it is possible that the hypothesis that the San Francisco Estuary is driven by phytoplankton production rather than through detrital pathways may have been accepted too strictly*." (Baxter et al. 2008)[55]

[54] Müller-Solger, A.B., A.D. Jassby, and D.C. Müller-Navarra. 2002. Nutritional quality of food resources for zooplankton (*Daphnia*) in a tidal freshwater system (Sacramento-San Joaquin River Delta). Limnol. Oceanogr. 47:1468-1476.

[55] Baxter, R., R. Breuer, L. Brown, M. Chotkowski, F. Feyrer, M. Gingras, B. Herbold, A. Müller-Solger, M. Nobriga, T. Sommer, and K. Souza. 2008. Pelagic organism decline progress report: 2007 Synthesis of results. Interagency Ecological Program for the San Francisco Estuary.

Copepod	Diatom	Egg Prod.	Hatching Success	Normal Nauplii	Complete Develop.
Acartia tonsa	*Thalassiosira weissflogii*	–			–
	Thalassiosira pseudo nana	–			–
	Thalassiosira weissflogii	+	+		
	Chaetoceros affinis	–			
	Phaeodacylum tricornutum	–	–		
Acartia hudsonica	*Skeletonema costatum*	+			
Acartia clausi	*Thalassiosira rotula*	+	–		
Centropages typicus	*Thalassiosira rotula*	–	–		
Temora stylifera	*Thalassiosira rotula*	–		–	–
	Skeletonema costatum			–	–
	Phaeodactylum tricornutum			–	–
	Thalassiosira rotula	+	–		
	Thalassiosira weissflogii	+	–		
	Phaeodactylum tricornutum	–	–		
	Skeletonema costatum	–	–		
	Thalassiosira rotula	+	–		
Temora longicornis	*Thalassiosira rotula*				+
	Thalassiosira weissflogii				+
	Leptocylindricus danicus				+
	Skeletonema costatum				+
	Chaetoceros affinis				–
	Chaetoceros decipiens				–
	Chaetoceros socialis				–
	Thalassiosira rotula				–
	Thalassiosira pseudo nana				–
	Thalassiosira rotula	+	–		
	Thalassiosira weissflogii	+	–		
	Chaetoceros affinis	+	–		
	Leptocylindricus danicus	–	–		
	Skeletonema costatum	–	–		

Figure 9. Reproductive consequences of direct feeding on diatoms for Delta copepod taxa. Experiments listed used copepod species from the Delta or their cofamilials. Positive (green) and negative (red) outcomes are indicated for four measures of reproductive success in feeding experiments: egg production (clutch size), hatching success, normal nauplii, and complete development of nauplii. Data are from the review of Ianora & Miralto (2010)[56] and other published literature reviewed in Engle (2010)[57].

None of the publicly available research from the Delta includes direct evidence that nutrient ratios (NH4:NO3, N:P, etc.) influence the taxonomic composition of phytoplankton in the Delta.

None of the experimental work to date in the Delta provides direct evidence that current N:P or NH4:NO3 ratios in the SFE provide a competitive disadvantage to diatoms and a competitive advantage to blue-green algae and flagellates. None of the publicly available research from the Delta has measured *taxon-specific* growth responses when phytoplankton assemblages were presented with different nutrient ratios in growth media. Microscopic identifications and cell counts, or other direct evidence of species composition, have not been reported for experimental manipulations of the NH4:NO3 ratio (such as the grow-out experiments conducted in Wilkerson et al. 2006, Dugdale et al. 2007, Parker et al. 2010). N:P ratios were not experimentally manipulated or compared to growth rates of different phytoplankton species in any Delta research cited in the ANPR.

There is no scientific evidence or consensus that N:P ratios are currently out of alignment in the Delta, or that lowering the N:P ratio would be beneficial for the Delta. There is no evidence that nitrogen and phosphorus are out of "stoichiometric" balance in the Delta. Deviations in atomic

[56] Ianora and Miralto (2010), *supra*, note 49
[57] Engle (2010), *supra*, note 52

TN:TP ratios in water samples from the classic "Redfield Ratio" of 16:1 (named for the oceanographer who determined in 1934 that the mean atomic N:P ratio of marine phytoplankton is 16:1 when neither nutrient limits growth) are often used as a rough indicator of relative N- or P- limitation of phytoplankton growth. Modern surveys indicate that TN:TP <18-22 may indicate N limitation in freshwater and ocean settings; phosphorus limitation is generally not expected unless TN:TP ratios exceed 50:1 (Guilford & Hecky 2000).[58] Boynton et al. (2008)[59] show that TN:TP ratios for 34 coastal, estuarine, and lagoon ecosystems trend somewhat above 16:1. Monthly samples for three IEP Suisun Bay monitoring stations for 2002-2007 provides a mean atomic TN:TP ratio of about 17:1 (16.7:1; Engle *unpublished* data[60]). This ratio is very close to the classic "Redfield Ratio." Lower ratios would be considered by many investigators as potential indicators of relative nitrogen deficiency in the water column. Significant concern exists regarding the low productivity of the Delta (Baxter et al. 2007),[61] and currently only a small fraction of in-Delta freshwater phytoplankton production escapes loss processes such as burial, in-Delta grazing, direct export in water diversions, to be transported into the brackish Delta (confluence zone and Suisun Bay) where the early life stages of POD fishes rear (Jassby et al. 2002).[62] Because there is experimental evidence from Parker et al. (2010) that Sacramento River phytoplankton entering the Delta upstream from the SRWTP are nitrogen-limited (see above), it is reasonable to predict that reductions in inorganic nitrogen might lower primary productivity in the Sacramento River.

The relationships between cellular indicators of nitrogen or phosphorus deficiency, inorganic nutrient concentrations, phytoplankton taxonomy and stoichiometry, and TN:TP ratios have not been studied in the SFE. In other words, *bona fide* research which would be required to determine whether current N:P ratios encourage or discourage the growth of particular phytoplankton taxa, or are in any way detrimental to the food web, has not been conducted in the Delta or the rest of the San Francisco Estuary. Central Valley Regional Board staff have acknowledged in 2010 that no science supports a "target" N:P ratio for the Delta:

> "*At this time there is no science to support what [N:P] ratio would be appropriate for the Sacramento River and the Sacramento-San Joaquin Delta*."[63]

There is also no scientific consensus that low N:P ratios favor diatoms over other phytoplankton groups. In fact, low N:P ratios (below the Redfield Ratio) are associated with a shift from diatoms to dinoflagellates in several estuaries[64]—a relationship which is opposite from that proposed for the Delta by some investigators.

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[58] Guildford, S.J., and R.E. Hecky. 2000. Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? Limnology and Oceanography 45:1213-1223.
[59] Boynton, W.R., J.D. Hagy, J.C. Cornwell, W.M. Kemp, S.M. Greene, M.S. Owens, J.E. Baker, and R.K. Larsen. 2008. Nutrient budgets and management actions in the Patuxent River Estuary, Maryland. Estuaries and Coasts. DOI 10.1007/s12237-008-9052-9.
[60] Data used for this calculation is available upon request and will be attached to SRCSD's April 25, 2011 letter to USEPA on the Advanced Notice of Proposed Rulemaking.
[61] Baxter, R., R. Breuer, L. Brown, M. Chotkowski, F. Feyrer, M. Gingras, B. Herbold, A. Müller-Solger, M. Nobriga, T. Sommer, and K. Souza. 2008. Pelagic organism decline progress report: 2007 Synthesis of results. Interagency Ecological Program for the San Francisco Estuary.
[62] Jassby, A.D., J.E. Cloern, B.E. Cole. 2002 Annual primary production: patterns and mechanisms of change in a nutrient-rich tidal estuary. Limnol Oceanogr 47:698-712.
[63] Staff Response to Comments, Regional Water Quality Control Board, Central Valley Region Board Meeting – 9 December 2010 Response to Written Comments for Sacramento Regional County Sanitation District Sacramento Regional Wastewater Treatment Plant Tentative Waste Discharge Requirements, p. 31.
[64] Hodgkiss, I.J., and K.C. Ho. 1997. Are changes in N:P ratios in coastal waters the key to increased red tide blooms? Hydrobiologia 352:141-147.

Potential negative ramifications of lowering N:P should be considered. For example, the competitive advantage of nuisance species of N-fixing cyanobacteria (e.g., *Aphanizomenon* and *Anabaena*) can increase in estuaries when N:P ratios are reduced if overall nutrient supplies are decreased and if seed populations are present (Piehler et al. 2002);[65] both taxa are present in the upper SFE.[66] Low N:P ratios can also induce blooms of the toxic alga *Microcystis* from resting stages in sediment (Stahl-Delbanco et al. 2003);[67] and, N:P ratios below the Redfield Ratio (i.e., <16:1) increase the risk of toxic red-tides in estuaries (Hodgkiss & Ho 1997).[68]

Table 1. Optimal N:P ratios promoting growth of toxic red tide causing organisms.*

Red Tide Causing Organism	Optimal N:P ratio for Growth	Optimal Ratio is below the Redfield Ratio?
Alexandrium catenella	15-30:1	sometimes
Ceratium furca	12-22:1	sometimes
Skeletonema costatum	15-30:1	sometimes
Gonyaulax polygramma	4-8:1	yes
Gymnodinium nagasakiense	11-16:1	yes
Noctiluca scintillans	8-14:1	yes
Prorocentrum dentatum	6-13:1	yes
Prorocentrum minimum	4-13:1	yes
Prorocentrum sigmoides	4-15:1	yes
Prorocentrum triestrium	8-15:1	yes
Scrippsiella trochoidea	6-13:1	yes

*Data are from Hodgkiss & Ho (1997).

Glibert (2010) used an improperly applied statistical transformation (CUSUM) to produce artificial and highly misleading correlations between nutrient parameters and biological parameters in the Delta. The Water Quality Findings on Nutrients cites Glibert (2010)[69] as evidence that total ammonia nitrogen loadings are correlated with the decline of pelagic fish or copepods in the Delta. Unfortunately, Glibert arrived at her conclusions using an improperly applied statistical transformation (cumulative sums of variability, or CUSUM) to produce artificial and highly misleading correlations between nutrient parameters and biological parameters (phytoplankton, zooplankton, fish abundance).

Glibert's approach is analytically and conceptually flawed, as detailed in Engle & Suverkropp

[65] Piehler, M.F., J. Dyble, P.H. Moisander, J.L. Pinckney, and H.W. Paerl. 2002. Effects of modified nutrient concentrations and ratios of the structure and function of the native phytoplankton community in the Neuse River Estuary, North Carolina, USA. Aquatic Ecology 36:371-385.

[66] Species belonging to the genera *Anabaena* an *Aphanizomenon* are on the list of species from IEP phytoplankton monitoring data in the upper SFE.

[67] Stahl-Delbanco, A., L. Hansson, and M. Gyllstrom. 2003. Recruitment of resting stages may induce blooms of Microcystis at low N:P ratios. J. Plank. Res. 25:1099-1106.

[68] Hodgkiss and Ho (1997), *supra, note 63*

[69] Glibert, P.M. (2010) Long-Term Changes in Nutrient Loading and Stoichiometry and Their Relationships with Changes in the Food Web and Dominant Pelagic Fish Species in the San Francisco Estuary, CA. Rev. Fish. Sci. 18:2, 211-232.

(2010)[70]. Further, the type of correlation analysis used in Glibert's article (cumulative sums of variability, or CUSUM) violates the underlying assumptions for linear regression and produces misleading results, which are not supported by underlying data. Other concerns include the limited geographic extent of the data, possible improper sub-sampling of CUSUM time series, nontransparent data reduction, and omissions of key analyses which were needed to support a claim for a link between nutrient ratios and the food web or which would support alternative hypotheses. Examples of these defects are summarized below:

Inadequate Geographic Coverage. Sweeping generalizations are made in Glibert's paper regarding the estuarine food web and the Pelagic Organism Decline (POD) using data from only one station in the Freshwater Delta (Hood, IEP station C3) and two stations in Suisun Bay (IEP stations D8 and D7).

Violation of Statistical Assumptions. Glibert used a calculation termed *CUSUM* to transform long-term datasets for nutrient concentrations and abundances of selected aquatic organisms, and then performed linear regression using the unordered transformed data for selected pairs of variables. Time series of CUSUM values exhibit features and patterns that diverge in several important ways from those of the underlying measured data and make them inappropriate for standard linear regression. CUSUM series mute seasonal or other short-term variations in a time series (which are important for short-lived organisms like phytoplankton and zooplankton), but exaggerate shifts that occur on long time scales (such as decades). In the statistical literature, CUSUM is primarily used to create charts (or ordered values) for single variables that allow the user to detect change points or determine whether deviations from control points are random or signal a trend. However, the characteristics of CUSUM that lend it useful to change-point analysis and quality control make it completely inappropriate to perform standard linear regression using paired CUSUM values removed from their respective temporal sequences.

Accordingly, the simple CUSUM correlations that represent the basis for Glibert's conclusions violate virtually every assumption of a standard correlation analysis. CUSUM series are inherently serially correlated, heteroscedastic and non-normally distributed, and the residuals of CUSUM correlations are non-independent.[71] Further, not all of the datasets used by Glibert are appropriate for customary uses of CUSUM. Autoregressive time series such as flow data are not appropriate for CUSUM change-point analysis. CUSUM change point analysis also assumes that underlying data are homoscedastic and often assumes that data are normally distributed. Glibert did not test raw data for autocorrelation, normality, or equal variance prior to the CUSUM transformation. Another requirement of CUSUM analysis is that time series being compared must start and stop at the same point in time. However, Glibert's correlations appear to be performed by pairing CUSUM series generated by underlying data spanning different time periods.

Artificial Relationships and Inflated R^2 Values. The CUSUM transformation results in a very

[70] Engle, D. and C. Suverkropp. 2010. Memorandum: Comments for Consideration by the State Water Resources Control Board Regarding the Scientific Article *Long-term Changes in Nutrient Loading and Stoichiometry and their Relationships with Changes in the Food Web and Dominant Pelagic Fish Species in the San Francisco Estuary, California* by Patricia Glibert. 17 pp. July 29, 2010.
[71] See Engle & Suverkropp (2010), *supra*, note 69, for more detail.

limited range of serially correlated data structures, which (if linear regression is performed for pairs of CUSUM series) leads to "correlations" with impressively inflated R^2 values that are largely artificial and cannot be interpreted in the same way as standard parametric correlation or regression analysis. Equally important, statistically significant relationships that *are* present in underlying data can be disguised when CUSUM time series are compared instead of real world measurements.

Nutrient Ratios were Not Compared to Biota at the Bottom of the Food Web. Despite widespread public perception to the contrary, Glibert failed to relate trends in nutrient ratios to those of phytoplankton or copepods in her article. Several obvious pairings of environmental variables were omitted from Glibert's portfolio of CUSUM correlations, including those that were needed for her to claim that nutrient ratios and phytoplankton taxa were statistically related. For example, CUSUM regressions between nutrient *ratios* (TN:TP, NO_3:NH_4, or DIN:DIP) and phytoplankton indices (chlorophyll-a or abundances of individual taxonomic groups) were not included in her analysis. Also, CUSUM trends in nutrient ratios were not directly compared to those for copepod abundance. NO_3:NH_4 trends were not compared to *any* of the biological trends (phytoplankton, copepods, clams, or fish); they were only compared to trends in Delta outflow. As a consequence, Glibert's publication did not make the case (even accepting its flawed statistical approach) that N:P ratios and phytoplankton composition are statistically related to each other, nor that N:P ratios are related to other abundances of other organisms (copepods) near the base of the pelagic food web in the Delta. In addition, the Glibert article reviewed no direct experimental evidence from the SFE or other systems that supports her conclusions regarding nutrient ratios and estuarine phytoplankton composition [or fish species populations?].

Glibert's selection of environmental parameters was biased, and did not include water. Glibert did not utilize data for export volumes as an independent variable in any of her CUSUM correlations. However, Figure 10 shows that when subjected to the same analysis used in Glibert's paper, annual water exports perform as well as ammonia concentrations in explaining trends in the summertime abundance of delta smelt. In addition to water export volumes, many other widely accepted alternative potential drivers of the changes in plankton composition or biomass and fish abundance in the SFE (and in estuaries, generally)—which would have been testable using her CUSUM methodology—were omitted from Glibert's analysis and from discussion in her article. Due to the peculiarity of the CUSUM transformation, it is likely that a wide variety of non-nutrient environmental factors (essentially any factors which have trended over time in the SFE in concert with changes in fish abundance such as clam abundance, invasive aquative macrophyte abundance, other invasive species abundances, turbidity, water exports, etc.) could be shown to be highly correlated with pelagic fish abundance using CUSUM correlations. For example, although Glibert's CUSUM correlations between fish abundance and ammonia are convenient for focusing attention on ammonia (as opposed to other potential drivers of the food web or the POD), they ultimately signify little with respect to the relative importance of multiple environmental factors, which have changed over recent decades in the SFE.

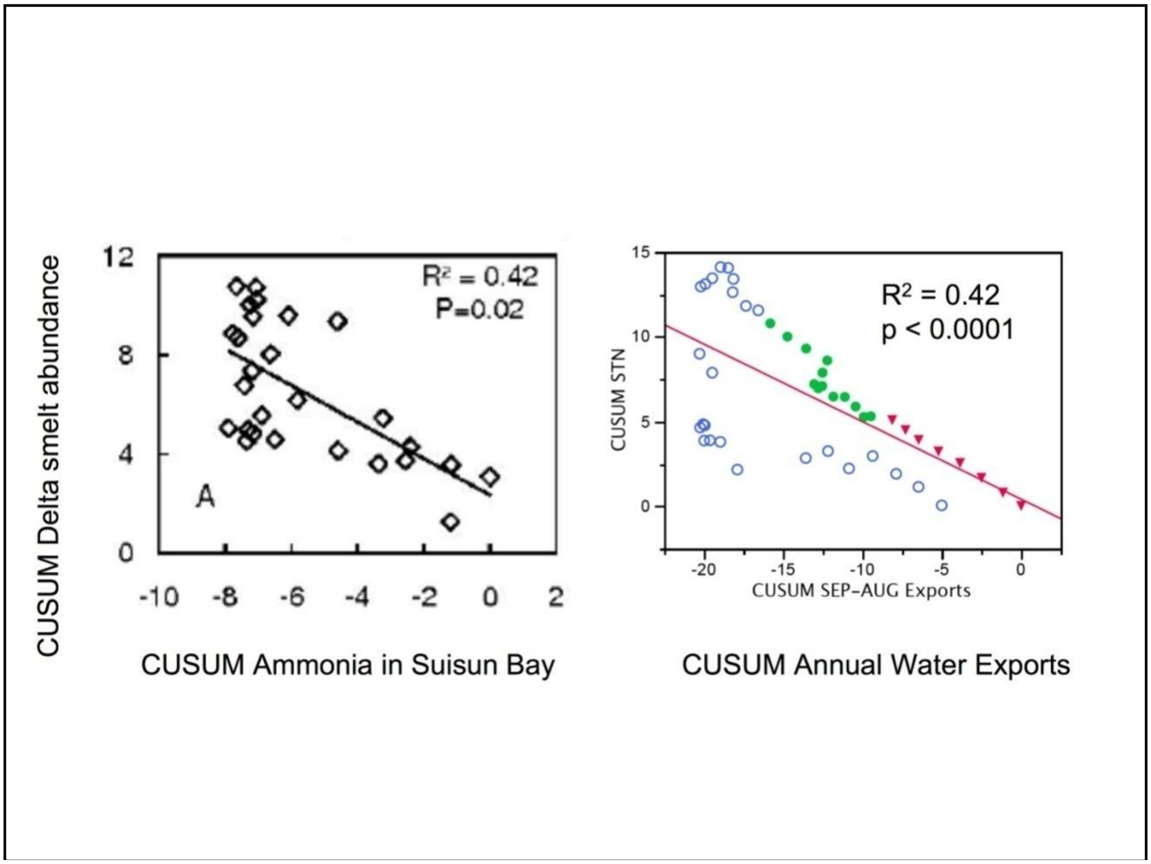

Figure 10. Comparison of correlations using CUSUM ammonia (Suisun Bay) or CUSUM annual Delta water exports (SWP, CVP, and Contra Costa Canal combined) as the independent variables (x-axis) and CUSUM values for the delta smelt Summer Townet Index as the dependent variable (y-axis). Correlation using ammonia is from Glibert (2010) and used data for 1975-2005. Correlation using annual water exports is from Engle & Suverkropp (2010)[72]; color coding for subsets of the CUSUM series is as follows: open blue circles for pre-Corbula years (1956-1986), solid green circles for post-*Corbula* years 1987-1999, red triangles for POD years 2000-2007. Details regarding underlying analyses are in Engle & Suverkropp (2010). The correlation coefficient (R^2 value) is the same for both regressions (0.42); both regression lines are significant. Figure is a combination of Figures 3 and 4 in Engle & Suverkropp (2010).

[72] Engle & Suvercropp (2010), supra, note 69